PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 April 1999 (09.04.99)	
International application No. PCT/CA98/00908	**Applicant's or agent's file reference** CG/10857.228
International filing date (day/month/year) 28 September 1998 (28.09.98)	**Priority date** (day/month/year) 30 September 1997 (30.09.97)
Applicant BKAILY, Ghassan et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 March 1999 (17.03.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

LECLERC, Alain M.
GOUDREAU GAGE DUBUC & MARTINEAU WALKER
Stock Exchange Tower
800 Place Victoria, Suite 3400
P.O. Box 242
Montreal, Quebec H4Z 1E9
CANADA

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	20.01.2000
Applicant's or agent's file reference CG/10857.228	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA98/00908	28/09/1998	30/09/1997

Applicant
UNIVERSITE DE SHERBROOKE et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	DA ROCHA, O. Tel.+49 89 2399-2735

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
GOUDREAU GAGE DUBUC & MARTINEAU WALKER
Attn. LECLERC, A.
Stock Exchange Tower
800 Place Victoria, Suite 3400
Montreal, Quebec H4Z 1E9
CANADA

Date of mailing (day/month/year) 18/02/1999

Applicant's or agent's file reference
CG/10857.228

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/CA 98/ 00908

International filing date (day/month/year) 28/09/1998

Applicant
UNIVERSIT DE SHERBROOKE et al.

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Heike Zoglauer

Form PCT/ISA/220 (January 1994)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
CG/10857.228		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 98/ 00908	28/09/1998	30/09/1997
Applicant		
UNIVERSIT DE SHERBROOKE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

Inte[redacted]nal Application No
PCT/CA 98/00908

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/58 C07J71/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	NEVES P C; NEVES M C; CRUZ A B; SANT'ANA A E; YUNES R A; CALIXTO J B: "Differential effects of Mandevilla velutina compounds on paw oedema induced by phospholipase A2 and phospholipase C. " EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 243, no. 3, 1993, pages 213-9, XP002092176 see the whole document --- -/--	1-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bardili, W

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BENTO, EDSON S.; CALIXTO, JOAO B.; HAWKES, GEOFFREY E.; PIZZOLATTI, MOACIR G.; SANT'ANA, ANTONIO E. G.; YUNES, ROSENDO A.: "The structure of velutinol A is (15R,16R,20S)-14,16:15,20:16,21-triepoxy-15,16-seco-14.beta.,17.alpha.-pregn-5-ene-3.beta.,15-diol. A combined quantitative Overhauser effect and molecular modeling study" J. CHEM. SOC., PERKIN TRANS. 2, no. 7, 1996, pages 1359-1366, XP002092177 see the whole document ---	1-17,26, 27
Y	EP 0 359 310 A (PROCTER & GAMBLE) 21 March 1990 cited in the application see page 1 - page 2 ---	1-27
X	BEYER: LEHRBUCH DER ORGANISCHEN CHEMIE, 1981, page 658 XP002092178 see 'pregnenolon' -----	9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/00908

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0359310 A	21-03-1990	JP 2142736 A	31-05-1990

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CG/10857.228		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA98/00908	28/09/1998	30/09/1997
International Patent Classification (IPC) or national classification and IPC A61K31/58		
Applicant UNIVERSITE DE SHERBROOKE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/03/1999	20.01.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bardili, W Telephone No. +49 89 2399 2132

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA98/00908

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-64 as originally filed

Claims, No.:

1-27 as originally filed

Drawings, sheets:

1/56-56/56 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-27
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-27
Industrial applicability (IA)	Yes:	Claims	1-17,26,27
	No:	Claims	18-25

2. Citations and explanations

see separate sheet

Section V:

Claims 1 to 10, 26 and 27 relate to naturally occurring compounds isolated from Mandevilla velutina and their analogues. In particular, compound MV 8608 and MV 8612 are claimed. Claims 11 to 25 pertain to pharmaceutical compositions comprising the compounds and their use in treating diseases related to an overstimulation of R-type Ca channels such as inflammatory diseases.

D1/ Eur. J. Pharmacol. 243, 213-9 (1993) discloses the isolation and anti-inflammatory activities of MV 8608 and MV 8612. The citation also mentions pharmaceutical compositions comprising the compounds and their potential in treating inflammation. Thus, claims 1 to 27 lack novelty.

The applicants' attention is also directed to D2/ J. Chem. Soc. Perkin Trans. 2, 1359 (1996) which discloses some of the structures claimed in claim 9.

For the assessment of the present claims 18 to 25 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

09/509462

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/58, C07J 71/00

A1

(11) International Publication Number: **WO 99/16449**

(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/CA98/00908

(22) International Filing Date: 28 September 1998 (28.09.98)

(30) Priority Data:
2,217,088 30 September 1997 (30.09.97) CA

(71) Applicant *(for all designated States except US)*: UNIVERSITE DE SHERBROOKE [CA/CA]; 2500, boulevard de l'Université, Sherbrooke, Québec J1K 2R1 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BKAILY, Ghassan [LB/CA]; 1840, rue Simard, Sherbrooke, Québec J1J 3X6 (CA). D'ORLEANS-JUSTE, Pedro [CA/CA]; 1709, rue Marcil, Sherbrooke, Québec J1J 2H7 (CA). CALIXTO, Joao, B. [BR/BR]; Rua Dr. Joao Carlos Baron Maurer, 40, Jardim Anchieta, CEP-88000-120 Florianopolis, SC (BR). YUNES, Rosendo, A. [BR/BR]; Rua Joaquim Costa, 185, Agronomica, CEP-88025-400 Florianopolis, SC (BR).

(74) Agents: DUBUC, Jean, H. et al.; Goudreau Gage Dubuc & Martineau Walker, The Stock Exchange Tower, Suite 3400, 800 Place Victoria, P.O. Box 242, Montreal, Quebec H4Z 1E9 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: *SPECIFIC* STEADY-STATE R-TYPE Ca^{2+} CHANNEL BLOCKERS AND USE THEREOF

(57) Abstract

The present invention relates to Ca^{2+} channel blockers and more particularly to the R-type Ca^{2+} channel blockers. More specifically, the invention relates to Ca^{2+} channel blockers activity of *Mandevilla velutina* and *Mandevilla illustris*. The present invention further concerns saponin-like compounds isolated from *Mandevilla* species. The present invention also relates to the treatment of several pathologies that involve the nifedipine-insensitive but isradipine sensitive steady-state R-type Ca^{2+} channel and the use of steady-state R-type Ca^{2+} channel blockers in the treatment of these pathologies.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
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CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TITLE OF THE INVENTION

SPECIFIC STEADY-STATE R-TYPE CA^{2+} CHANNEL BLOCKERS AND USE THEREOF.

FIELD OF THE INVENTION

The present invention relates to Ca^{2+} channel blockers and more particularly to the R-type Ca^{2+} channel blockers. More specifically, the invention relates to Ca2+ channel blockers activity of *Mandevilla velutina* and *Mandevilla illustris*. The present invention further concerns saponin-like compounds isolated from *Mandevilla* species. The present invention also relates to the treatment of several pathologies that involve the nifedipine-insensitive but isradipine sensitive steady-state R-type Ca^{2+} channel and the use of steady-state R-type Ca^{2+} channel blockers in the treatment of these pathologies.

BACKGROUND OF THE INVENTION

Sustained increase of intracellular Ca^{2+} or sustained Ca^{2+} overload (cytosolic, nuclear and mitochondrial) is known to be associated with many abnormal cell function including hypertension, artherosclerosis, hyperinsulinemia, diabetes Melitus type II, abnormal cell proliferation, cell-cell interactions, necrosis, ischemia/reperfusion, arrythmias, platelet activation and aggregation as well as inflammation and asthma (Bkaily, 1994, Medical Intelligence Unit, CRC Press, Austin; Bkaily and Jacques, 1994, Kluwer Academic Publ., Boston; Bkaily et al., 1994, Kluwer Academic Publ., Boston; Bkaily et al., 1997, Can. J. Physiol. Pharmacol. 75:652-666; Bkaily et al., 1997, Mol. Cell. Biochem. 172:171-194; Bkaily et al., 1997b, Drug Devel. Res. 42:211-222; Sowers et al., 1993, Am. J. Hypert. 6:302-307; 1994; Hurwitz et al., 1991, CRC Press, Boca Raton, Ann Arbor; Nagano et al., 1992, Kluwer Academic Publ., Boston; Anand et al., 1989, Kluwer Academic Publ. Boston; Dhalla et al., 1996, Kluwer Academic Publ. Boston; Karmazyn, 1996, Birkhauser Verlag. Basel, Boston; Curtis, 1993, Academic Press. London, San Diego; De Brum et

al., 1996, Br. J. Pharmacol. 118:1597-1604; Foreman, 1993, Academic Press, London, San Diego; Furberg et al., 1993, Am. J. Hypert. 6:24S-29S; Holgate et al., 1993, Academic Press, London, Boston; Jacobs et al., 1993, Hypertension 21:308-314; Johnson et al., 1993, J. Clinic. Pharmacol. 35:484-492; Levy et al., 1994, Am. J. Med. 96:260-273; Raman et al., 1995, Am. J. Hypert. 8:197-200; Sperelakis et al., 1984, Martinus Nijhoff Publ. Boston; Standley et al., 1993; Wray et al., 1989, The New York Academy of Sciences, New York. Vol. 560). A wide variety of drugs has been tested against different types of Ca^{2+} channels (P, N, T and L) and the development of Ca^{2+} blockers has been concentrated on the L-type Ca^{2+} channel, which has never been shown to undergo any abnormal function in many diseases implicating sustained increase of intracellular Ca^{2+} ($[Ca]_i$) or Ca^{2+} overload. Also, these drugs with the exception of isradipine (PN200-110, Lomir/Dynacirc), failed to block or prevent the sustained increase of $[Ca]_i$, Ca^{2+} overload and necrosis. Recently, the presence of a steady-state nifedipine (L-type blocker)-insensitive but isradipine sensitive (dual L and R-type blocker) R-type (resting-type) Ca^{2+} channel that is voltage and Ligand-G protein-dependent has been reported (Bkaily et al., 1991, Elsevier, New York; Bkaily et al., 1992a, Am. J. Physiol. 262:H463-471; 1993, Br. J. Pharmacol. 110:519-520; 1993a, J. Mol. Cell. Cardiol. 25:1305-1316; 1995, J. Cardiovascul. Pharmacol. 26:303-306; 1996, Mol. Cell. Biochem. 154:113-121; 1997, Drug Develop. Res. 42:211-222; 1997a, Mol. Cell. Biochem. 172:171-194; 1997b, Can. J. Physiol. Pharmacol. 75:652-666; 1997d, Mol. Cell. Biochem. 170:1-8; 1997d, Mol. Cell. Biochem. 176:199-204; 1998, Mol. Cell. Biochem., 183:39-47; Bkaily, 1994a, In: Ionic channels in vascular smooth muscle. G. Bkaily edt. Molecular Biology Intelligence Unit, R.G. Lands Co. Austin.). This channel was responsible for maintaining the resting cytosolic and nuclear Ca^{2+} levels and its overstimulation by sustained depolarization or by permanent presence of some hormones such as insulin, ET-1, PAF, TNFα, PDGF, Bradykinin, or IL-1 induced sustained increase of $[Ca]_c$ and $[Ca]_n$. (Bkaily et al., 1991, Elsevier, New York; 1993, *supra*; 1995, *supra*; 1996, *supra*, 1997a, *supra*; 1997b, *supra*; 1997c,

supra; 1997d, *supra*; 1998, *supra*; Bkaily, 1994a, *supra*; Bkaily, 1994b, In: Membrane physiopathology. G. Bkaily edt. Kluwer Acad. Publ. Boston; Taoudi et al., 1995, J. Cardiovasc Pharmacol. 26:300-302). The important features that distinguish this channel from other Ca^{2+} channels are the sustained activity (as long as a depolarization or the pharmacological and physiological agonist is present) and the large number of disparate agonists that indirectly (via receptor-G proteins coupling) stimulate the channel.

Several reviews described the presence of various types of voltage-dependent Ca^{2+} channels in many cell types including heart, vascular smooth muscle (VSM) and vascular endothelial (VE) cells (Godfraind and Govoni, 1995; Bkaily, 1994b, *supra*; Orallo, 1996, Bkaily et al., 1997a, *supra*). Among these different types of Ca^{2+} channels, the resting membrane potential steady-state voltage-dependent R-type (for resting) Ca^{2+} channel was first reported by the group of Bkaily et al. (Bkaily et al., 1991, *supra*; 1992, *supra*; Bkaily, 1994, *supra*). Later, the group of Tsien (Zhang et al., 1993, Neuropharmacol 32:1075-1088; Randall et al., 1997, Neuropharmacol 36:879-893) described a dihydropyridine resistant type Ca^{2+} channel also named R-type (for resistant).

The steady-state R-type Ca^{2+} channel in human VSM and VE cells was reported to possess a nearly 24 pS single channel conductance (in 110 mM Ca^{2+}) (Bkaily et al., 1997a, *supra*). This type of channel was shown to be responsible for determining, under normal conditions, the resting tension of VSM cells and secretions by VE cells (Bkaily et al., 1991, *supra*; Bkaily et al., 1992, *supra*; Bkaily et al., 1993, *supra*; Bkaily et al., 1995, *supra*; Bkaily et al., 1996; Bkaily et al.,1997a; Bkaily et al., 1997b; Claing et al., 1994, Br. J. Pharmacol. 112:1202-1208; Taoudi-Benchekroun et al., 1995, J. Cardiovasc Pharmacol. 26:300-302). This type of Ca^{2+} channel is known to be insensitive to nifedipine and inorganic L-type Ca^{2+} channel blockers such as cobalt, cadmium and Mn^{2+} and the T-type Ca^{2+} blocker, nickel (Bkaily, 1994, *supra*). However, it is blocked by PN200-110 (isradipine) which is also known to block the L-type

Ca^{2+} channel (Bkaily et al., 1992, *supra*; Bkaily et al., 1997a, *supra*). Unlike T and L-type Ca^{2+} channels, the R-type Ca^{2+} channel was not regulated by second messengers such as cAMP, cGMP and protein kinase C and neither by ATP (Bkaily, 1994, *supra*). This type of channel was reported to be indirectly stimulated by insulin, PAF, ET-1 and bradykinin via stimulation of a PTX and CTX sensitive G-protein(s) (Bkaily, 1994a, *supra*; 1991, *supra*; 1992, *supra*; 1995; 1996, *supra*; 1997a, *supra*; 1997b, *supra*; 1997c, *supra*; 1998, *supra*) and to contribute to a sustained elevation of cytosolic ($[Ca]_c$) and nuclear ($[Ca]_n$) Ca^{2+}. These indirect R-type Ca^{2+} channel stimulators such as PAF induced elevation of $[Ca]_c$ and $[Ca]_n$ by increasing the probability of opening of the channel, and thus allowing longer influx of Ca^{2+} through the sarcolemmal membrane (Bkaily et al., 1997a, *supra*).

Since PN200-110 was found to be the only available compound to depress the R-type Ca^{2+} channel and since this Ca^{2+} blocker is known to affect other types of Ca^{2+} channels, there remains a need to develop specific and potent steady-state R-type Ca^{2+} channel blockers.

The steady-state R-type Ca^{2+} channels are distributed in a non-homogenous fashion, similarly to some other receptors. This type of channel seems to have no inactivation gate and it is highly selective for Ca^{2+} ions. The R-type Ca^{2+} channel is highly voltage-dependent but could be stimulated by receptors whose activation is coupled to a specific PTX and CTX-sensitive G-protein(s) (Bkaily et al., 1998, *supra*). Thus, if the R-type Ca^{2+} channel is fully activated via a receptor dependent pathway, it may appear as a receptor operated Ca^{2+} channel. Moreover, if the R-type channel is fully activated by voltage, receptor stimulation does not further modulate its function and appears as a pure voltage-dependent channel (Bkaily, 1994, *supra*; 1997a, *supra*; 1997c, *supra*). Since T- and L-type Ca^{2+} channels are rapidly inactivated during sustained voltage or pharmacological stimulation, these types of channels can only contribute to the inset stimulation. However, the R-type Ca^{2+} channel will contribute to both inset and sustained elevation of cytosolic and

nuclear free Ca^{2+}, seen in normal and pathological conditions; depending on the function of the studied cell type. Hence, this type of channel, under normal physiological situations contributes to the resting Ca^{2+} influx responsible for determining the resting cytosolic and nuclear Ca^{2+} that modulate resting tension, secretion, protein synthesis and mitosis. In working muscle cells, such as heart cells, the normal physiological function of this channel at the sarcolemmal membrane level, is to maintain normal resting cytosolic Ca^{2+} level. However, at the nuclear membrane levels, this channel seems to be implicated in maintaining normal resting nucleoplasmic Ca^{2+} levels (near 300 nM) (Bkaily et al., 1997a, *supra*; 1997b, *supra*).

During excitation-contraction coupling, the R-type Ca^{2+} channel is implicated in regulatingCa^{2+} wave propagation initiated, by Ca^{2+} influx through the opening of the T- and L-type Ca^{2+} channels and the subsequent large Ca^{2+} release from the SR by attenuating the cytosolic Ca^{2+} wave amplitude, by allowing Ca^{2+} influx through the nuclear membrane and thus permitting a smooth contraction and relaxation. The subsequent release of the uptaken Ca^{2+} permits the maintainance of Ca^{2+} waves and slow relaxation and propagation of the waves to neighboring cells, most likely through gap-junctions and in this manner, allowing synchronization of contraction of ventricular cells (Lopez et al., 1995, Biochem Biophys Res Commun 214:781-787; Bkaily et al., 1996, *supra*; 1997a, *supra*). The fact that cytosolic Ca^{2+} waves cannot be completely absorbed by the nucleus is due to the maximum Ca^{2+} buffering capacity of the nucleus which is shielded from variations in cytoplasmic Ca^{2+}, perhaps by gating mechanisms in the perinuclear envelope once its maximum capacity is reached (Burnier et al., 1994, Am J Physiol 266:C1118-C1127; Bkaily, 1994, *supra*; 1996, *supra*; 1997a, *supra*, 1997b, *supra*).

Recent published results also showed that in secretory cells such as VE and VSM cells, tonic secretion or contraction is mainly, if not only due to the activation of sarcolemmal R-type Ca^{2+} channels. It was further shown that in VSM and excitable cells (VE cells do not possess T- or L-type Ca^{2+}

channels) the T and/or the L-type Ca^{2+} channel activation, serves as a turbo Ca^{2+} influx mechanism in order to rapidly bring the Ca^{2+} level up to the threshold level for contractile elements and to pre-overload the nucleoplasm with Ca^{2+}, enabling cytosolic accumulation of Ca^{2+} and maintain tension. Thus, overstimulation of the R-type Ca^{2+} channels may highly contribute to cytosolic and nuclear Ca^{2+} accumulation that could be considered in many cases as the first and in all cases the final pathological consequence of several diseases such as hypertension, atherosclerosis, abnormal conduction, arythmias, fibrillation, and of remodelling, proliferation and apoptosis. For these reasons, targeting the sarcolemmal nuclear membrane R-type Ca^{2+} channels with a selective depressor blocker, or targeting receptors that indirectly modulate this type of channel at the sarcolemmal, or mainly at the nuclear membrane level, would constitute without any doubt, a major therapeutical pathway for a new generation of Ca^{2+} channel and Ca^{2+} entry blockers.

For example, the sustained activation of the R-type channel by insulin may explain in part " syndrome X ", the hypertension, hyperglycemia, dyslipidemia, vascular smooth muscle proliferation and end organ damage associated with non-insulin-dependent diabetes mellitus (NIDDM) and obesity-induced hypertension. Also, the sustained increase in $[Ca]_c$ and mainly $[Ca]_n$ mediated by the stimulation of the R-type Ca^{2+} channel could contribute to the expression of oncogenes and to the proliferation of malignant cells as well as to stimulation of TNFα; PAF which would lead to septic shock. The finding that Lomir/Dynacirc (but none of the other L-type Ca^{2+} antagonists) is unique in depressing the overstimulation of the R-type Ca^{2+} channel permits the identification and characterization of this type of Ca^{2+} channel. Non published results in two human osteoblast cancer lines (MG63 and FAOS-2) clearly showed that Lomir/Dynacirc (10^{-8}M) reduced spontaneous cell proliferation and blocked hypertension and ET-1 plasma elevation associated with cyclosporin A treatment in allograft transplant. In contrast, an L-type Ca^{2+} blocker, nifedipine (10^{-6}M) had no effect. A role for the R-type Ca^{2+} channel and Lomir/Dynacirc in

human cancer is suggested by the above findings and supported by the finding of reduced cancer rates in the Lomir/Dynacirc treated group of the MIDAS study. The identification of a potent and specific antagonists may hold the possibility of a new therapeutic target for novel medications. The novel R-type Ca^{2+} channel may also prove important in dissecting differential signalling pathways in immune cells. The evaluation of these mechanisms leads to R-type blockade as a therapeutic tool for specific intervention in graft rejection, autoimmune diseases, asthma and septic shock.

A recent report in patients with type I and type II Raynaud's phenomenon (pain and numbness in the fingers, which in some subjects can be complicated by skin ulcers) showed that Lomir/Dynacirc significantly reduced the elevated plasma concentration of ET-1 level, frequency, severity, and disabling nature of acute attacks of Raynaud's phenomenon (La Civita et al., 1996, Clinic. Drugs Invest. 11:S126-31). The decrease of the elevated ET-1 circulating level by Lomir/Dynacirc is due to the blockade of the R-type Ca^{2+} channel which reverses the sustained increase of $[Ca]_c$ and $[Ca]_n$, thus, reducing the autocrine and self perpetuating secretion of mitogenic factors such as ET-1, PAF and TNFα. A blockade of the elevated autocrine and self perpetuating secretion of mitogenic factors by cancer cells may in turn contribute to reduction and even blockade of expression of oncogenes and proliferation of these cells.

The use of Sandimmune is known to produce potentially serious side effects such as renal impairment and hypertension. These side effects will restrict Sandiummune's use in autoimmune indications such as psoriasis and rheumatoid arthritis. The renal impairment and hypertension are attributable to altered renal hemodynamics induced by Sandimmune. The L-type Ca^{2+}-channel blockers have been used successfully to treat hypertension and renal impairment. The benefits of the Ca^{2+}-channel blockers have been attributed to their effects on renal hemodynamics specifically dilation of the afferent renal arteriole.

Data indicate that the dual R-and L-type Ca^{2+} channel blocker isradipine (but not a pure L-type blocker) may correct the vasoconstriction at both the afferent and efferent renal arterioles. The advantage of dilation of the afferent and efferent arterioles is a correction of renal blood flow and glomerular filtration without an increase in filtration fraction. Filtration fraction is an indicator of filtration pressure. An increase in filtration pressure could increase the likelihood of developing glomerulonephritis and eventual renal failure.

The potential benefit of blockade of the R-type Ca^{2+} channel by Lomir/Dynacirc on filtration pressure is supported by the existing literature. For example Grossman et al. (1991, Am. J. Cardiol. 68:65-70) in a 3-month study with Lomir/Dynacirc showed that filtration fraction remained constant. The filtration fraction remained constant despite the increase in glomerular filtration rate and renal blood flow. Vascular resistance was also reduced by the 3-month treatment with Lomir/Dynacirc.

A favourable effect on filtration fraction has been corroborated in transplant patients (Berg et al., 1991, Nephrology, Dialysis, Transplantation. 6:725-30). These investigators showed that filtration fraction was reduced by Lomir/Dynacirc while renal blood flow increased.

The R-type Ca^{2+} channel has also been identified and characterised in vascular smooth muscle cells isolated from human renal arteries (Bkaily et al., 1991, *supra*).

Mandevilla velutina is a native Brazilian plant used in folk medicine to treat snake bites and as an anti-inflammatory agent. Some non-peptidic compounds extracted from this plant block bradykinin and related kinins action. It shows potent analgesic and anti-inflammatory activities (Calixto et al., 1987, *supra*).

Since 1985, Calixto's group has worked on extracts of *Mandevilla velutina* (MV) and claimed that some of the extracts (such as MV8608) had antagonistic properties against the effect of bradykinin (BK). The compound MV8608 has been characterized in 1987 (Calixto et al., Br. J.

Pharmacol. 91:199-204). It has been found to be selective in its ability to inhibit the contraction of rat uterus induced by BK. The previous work made by Calixto's group as well as others on extracts of Mandevilla species have always focused on compounds which have a presumed action at the BK receptor site.

In a review article published after 1990, Calixto's group (Calixto and Yunes, 1991, Mem. Inst. Oswaldo 86:195-202, supl. 2) mentioned that the compounds MV8608 had a pregnane structure. It is further mentioned that MV8608 is an aglycone compound (without any sugar). No specific structure is shown in this review article concerning MV8612. This review is a compendium of data, characteristics, and properties of MV8608 and MV8612 in numerous systems responding to BK (therefore not limited to the effect of BK on rat uterus). Again, it may be deducted from this publication that the Calixto group of researchers have focused their study on the search of a ligand which is a BK receptor antagonist. MV8612 has been retained as a good candidate because it best corresponds to established receptor classification criteria (a fairly good pA2, competition curve whose slope does not differ from one and selectivity). Of note, Calixto publication (Calixto and Yunes, 1991, *supra*) does not teach or suggest that MV8612 may have an action which is aimed at the receptor directly. Although on certain systems the effect of MV8612 has been shown to be non-selective, no explanation on this lack of selectivity toward BK has been provided. Therefore, this publication does not teach or suggest any role of MV8608 and MV8612 as calcium channel blockers.

Other compounds isolated from *Mandevilla Pentlandia*, have also claimed an anti-BK activity, (patent application of Proctor and Gamble Co., EP 0/359310). Furthermore, other *Mandevilla* extracts, particularly from *Mandevilla Illustris* have been shown to have physiological antagonist activity against BK. Indeed, all the compounds obtained from *Mandevilla* species are described as compounds having anti-BK activity. Strikingly, all such descriptions fail to teach or mention the specific site of action of these compounds, and while they lack selectivity, they are deemed to be useful for treating pathologies and

conditions involving bradykinin (inflammation, smooth muscle contraction, pain, hypotension, etc.).

The art teaches that a non-specific inhibitor of a calcium channel such as isradipine, which has an effect on calcium channel types L and R, reduces or abolishes the effect of hormones like insulin and PAF (platelet-activating factor), ET-1 and BK which effect is absent when using nifedipine (a L-channel blocker). Nevertheless, the art is indicative of the contribution of the R-type calcium channel in the effect of insulin, PAF, ET-1 and BK.

There thus remains a need to assess the specificity of MV8608 and MV8612 by identifying their direct or indirect effects on Ca^{2+} homeostasis. More broadly, there remains a need to verify whether MV8608 and MV8612 are as non-specific as isradipine. More particularly, there remains a need to assess the activity of these compounds on the R-type Ca^{2+} channel, as well as T, L Ca^{2+} channels and the fast Na^+ and delayed outward K^+ channels.

In spite of the recent discovery of the R-type Ca^{2+} channel, there is a definite need for a new generation of class of drugs to treat overstimulation of R-type Ca^{2+} channel-associated diseases for the following reasons:

1. There is no drug approved for the treatment of diseases or conditions in which a sustained elevation of $[Ca]_c$, $[Ca]_n$ or R-type Ca^{2+} blocking is encountered; and

2. There remains a definite need for the identification of drugs which are more specific, show less side effects and have a wider therapeutic value, for the treatment of hypertension, artherosclerosis, inflammation, septic shock, arthritis, asthma, cancer, pain, diabetes type II and ischemia-reperfusion, hyperventilation and high circulating ET-1 level.

The present invention seeks to meet these and other needs.

SUMMARY OF THE INVENTION

The invention concerns saponin-like compounds isolated from *Mandevilla* species. More specifically, the invention concern saponin-like compounds isolated from *Mandevilla* species which act as specific R-type Ca^{2+} channel blockers.

In a particular embodiment, the present invention relates to R-type Ca^{2+} channel blocker obtained from *Mandevilla* species and more particularly from *Mandevilla Velutina* and *Mandevilla Illustris*. In a preferred embodiment, the invention relates to specific R-type Ca^{2+} channel blockers MV8608 and MV8612.

The invention in addition relates to pharmaceutical compositions comprising specific R-type Ca^{2+} channel blockers obtained from *Mandevilla* species to treat and/or prevent diseases or conditions associated with a sustained elevation of $[Ca]_c$, $[Ca]_n$, or R-type Ca^{2+} blocking and/or cytosolic and nuclear Ca^{2+} accumulation, together with a suitable pharmaceutical carrier. More specifically, the present invention relates to such pharmaceutical compositions to treat or prevent hypertension, artherosclerosis, hyperinsulinemia, diabetes Melitus type II, abnormal cell proliferation, cell-cell interactions, necrosis, ischemia/reperfusion, arrhythmias, platelet activation and aggregation, inflammation, asthma, abnormal conduction, fibrillation, remodelling, proliferation, antibacterial proliferation, septic shock, apoptosis, hyperglycemia, dyslipidemia, vascular smooth muscle proliferation and end organ damage associated with non-insulin-dependent diabetes mellitus (NIDDM), and obesity-induced hypertension, cancer, renal impairment, renal failure, arthritis pain, hyperventilation, and high circulating ET-1 level.

The invention further relates to a family of R-type Ca^{2+} channel blockers which virtually only affect the overstimulation thereof, without significantly affecting the basal activity thereof.

In addition, the invention relates to a family of specific R-type Ca^{2+} channel blockers which reduce the over-basal frequency of the R-type Ca^{2+} channel.

The invention also relates to methods of preventing or treating a warm blooded animal having a disease or condition demonstrating a sustained elevation of calcium through an effect on a R-type Ca^{2+} channel, comprising an administration of an effective amount of specific R-type Ca^{2+} channel blocker, in accordance with the present invention, together with a pharmaceutically acceptable carrier. The invention also relates to pharmaceutical compositions for such methods of prevention or treatment.

The invention further relates to methods of treatment of a warm blooded animal in need of this treatment comprising an administration of a therapeutically effective amount of a R-type Ca^{2+} channel blocker obtained from *Mandevilla* species, together with a pharmaceutically acceptable carrier. More specifically, the present invention relates to a treatment of a warm blooded animal demonstrating a sustained elevation of $[Ca]_c$, $[Ca]_n$, R-type Ca^{2+} blocking, and/or cytosolic and nuclear Ca^{2+} accumulation, comprising an administration of a therapeutically effective amount of a R-type Ca^{2+} channel blocker obtained from *Mandevilla* species, together with an acceptable pharmaceutical carrier.

Before the present invention, the properties of the MV compounds towards the calcium channel type R disclosed had not been taught or suggested.

Furthermore, before the present invention, it was unknown that in organ transplants, in an animal model such as rabbit, that there is an increase of circulating ET-1 and a decrease of blood flow that were not prevented by cyclosporin-A and by the pure L-type blocker nifedipine. In contrast, the dual R- and L-type Ca^{2+} blocker Lomir/Dynacirc (isradipine or PN200-110), restored ET-1 and blood flow levels in cyclosporin-A treated and transplanted animals. Thus, the present invention shows that some undesired

side effects associated with cyclosporin-A treatment for example, are attributable to an overstimulation of R-type Ca^{2+} channels.

While a blockade of the elevated autocrine and self perpetuating secretion of mitogenic factors by cancer cells could have been suggested from the results by La Civita et al. (1996, *supra*), prior to the present invention, it was unknown whether this proliferative effect was related to the R-type Ca^{2+} channel and whether it was a common mechanism in cancer and tumor cells. In accordance with the present invention, preliminary results using several types of human cancer cell lines seem to highly suggest that the proliferative effect of overstimulation acts through the R-type Ca^{2+} channel and is indeed a common mechanism in cancer and tumor cells. Thus supporting the results for the R-type Ca^{2+} channel and its blockade by Lomir/Dynacirc in human cancer as mentioned above in the Lomir/Dynacirc group of the MIDAS cohort (a non-specific R-type and C-type channel blocker). Thus, the present invention relates to a method of decreasing proliferation of cancer and tumor cells comprising an incubation thereof with an effective amount of a R-type Ca^{2+} channel blocker. More particularly, the present invention relates to a method of decreasing proliferation of cancer and tumor cells comprising an incubation thereof with an effective amount of a R-type Ca^{2+} channel blocker obtained from *Mandevilla* species and even more particularly of MV8608 and MV8612. In addition, the present invention provides a method for preventing non-properly regulated autocrine secretion using the specific R-type Ca^{2+} channel blockers of the present invention (and pharmaceutical compositions therefor).

Further, prior to the present invention, it was unknown that a combination therapy of certain drugs which display potentially serious side effects such as renal impairment and hypertension (i.e. Sandimmune) with R-type Ca^{2+} channel blockers could block or relieve these side effects. Furthermore, data using the rabbit (see example 9) indicate the presence of the R-type Ca^{2+} channel in both the afferent and efferent renal arterioles. It remains to be seen whether the R-type Ca^{2+} channel is present in renal arterioles. The

instant invention along with that of all the published papers since 1991 by Bkaily et al. provides the rationale for an advantage of R-type Ca^{2+} blocking agent such as Lomir/Dynacirc over other pure L-type Ca^{2+} channel blockers in the long-term protection of renal function. The contribution of endothelin to the Sandimmune induced side effects of renal impairment and hypertension has now been assessed. The results highly suggest that blockade of the R-type Ca^{2+} channel by Lomir/Dynacirc blocks the elevated circulating endothelin level induced with the Sandimmune drugs (Bkaily et al., data not shown).

The compounds of the present invention, in contradistinction to nifedipine and isradipine, do not induce hypertension in a normal patient.

From the wide variety of L-type Ca^{2+} channel blockers such as nifedipine, nicardipine, Diltiazem, Clentiazem, Verapamil, D600 and D888 none were found to block the R-type Ca^{2+} with the exception of isradipine (Lomir/Dynacirc) (data not shown). This later compound was found to block the R-type Ca^{2+} channel with an ED_{50} near (10^{-8} M), the T-type Ca^{2+} channel with an ED_{50} near (10^{-7}M), the L-type Ca^{2+} channel with an ED_{50} near (10^{-9}M) and the fast Na^{+} channel with an ED_{50} near (10^{-5}M) (data not shown). Thus, although the R-type Ca^{2+} channel blocker isradipine seems to be a potent R-type Ca^{2+} blocker, it is not highly specific. On a clinical point of view, this drug seems to be more potent and to possess less side effects than other dihydropyridines (DHP) derivative L-type Ca^{2+} channel blockers. The difference between isradipine and other DHPs compounds could be due to the potential of the former, to act as a R-type Ca^{2+} blocker.

It has now been demonstrated that similarly to PAF, insulin and ET-1, bradykinin (BK) also induced a sustained increase of $[Ca]_i$ which was mainly nuclear and was due to the stimulation of the R-type Ca^{2+} channel in human aortic endothelial and vascular smooth muscle, as well as chick and heart ventricular cells. The stimulation of R-type Ca^{2+} channel by BK was due to the kinin activation of the B_1-receptor.

In accordance with the present invention, there is therefore provided a compound having the general formula of MV8612 analogs VIIA and VIIB, saponin-like derivatives thereof and pharmaceutically acceptable salts thereof.

In accordance with the present invention, there is also provided a saponin-like compound having the general formula EST or a derivative of the saponin-like compound, wherein E and S define a saponin oligosugar portion and T defines a steroid-like portion; wherein T is a pregnane-3β-ol derivative.

In addition, in accordance with the present invention, there is also provided a R-type Ca^{2+} channel blocker having the general formula defined herein as EST.

Further, in accordance with the present invention, there is provided a specific R-type calcium channel inhibitor having the general formula EST.

In accordance with the present invention there is also provided a pharmaceutical composition for treating or preventing overstimulation of R-type Ca^{2+} channels associated disease or condition in a warm blooded animal comprising at least one compound of general formula EST, together with a pharmaceutically acceptable carrier.

As well, in accordance with the present invention, there is also provided a pharmaceutical composition for blocking or relieving side effects of a drug which overstimulate R-type Ca^{2+} channels comprising at least one compound of general formula EST, together with a pharmaceutically acceptable carrier.

In accordance with the present invention, there is also provided a method for specifically inhibiting overstimulation of a R-type Ca^{2+} channel in a warm blooded animal comprising an administration of an effective amount of a compound of general formula EST,together with a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1 shows the structure of compound of MV8608 and its isomers; as well as the structure of 5-pregnane-3ß-ol-20 (compound V);

Figure 2 shows the structure of MV8612 (analogs VIIA and VIIB);

Figure 3 shows ^{1}HNMR of compound MV8612 isolated from *Mandevilla Velutina*;

Figure 4 shows ^{13}CNMR of compound MV8612 isolated from *Mandevilla Velutina*;

Figure 5 shows COSY (correlated spectroscopy) of compound MV8612 isolated from *M. Velutina*;

Figure 6 shows HETCOR of compound MV8612 isolated from *M. Velutina*;

Figure 7 shows HPLC chromatogram of compound MV8608 isolated from *M. Velutina*;

Figure 8 shows HPLC chromatogram of compound MV8612 isolated from *M. Velutina*;

Figure 9 shows GC chromatogram of compound MV8608 isolated from *M. Velutina*;

Figure 10 shows GC chromatogram of compound illustrol isolated from *M. Illustris*;

Figure 11 shows the absence of the effect of (10^{-7}M) of MV8608 on the TTX-sensitive fast Na^{+} current in single heart cell;

Figure 12 shows the relative weak depressing effect of (10^{-9}M) and (10^{-7}M) of MV8608 on the T-type Ca^{2+} current in heart cells;

Figure 13 shows the relative weak depressing effect of (10^{-9}M) and 5 x (10^{-7}M) of MV8608 on the L-type Ca^{2+} current in heart cells;

Figure 14 shows that intra patch pipette application of (10^{-7}M) of MV8608 decreased the R-type Ca^{2+} channel amplitude and probability of opening and that extra patch pipette application of MV8608 (10^{-7}M) increased the time of opening duration followed by transient decrease of the amplitude of the R-type Ca^{2+} channel;

Figure 15 shows the blockade by MV8608 (10^{-9}M) of the sustained depolarization induced by sustained increase of total $[Ca]_i$ via activation of the R-type Ca^{2+} channels in chick and human heart cells;

Figure 16 shows the absence of the effect of nifedipine (10^{-6}M) on ET-1 (10^{-9}M), sustained depolarization (KCl, 30 mM) and PAF (10^{-9}M) induced sustained increase of $[Ca]_i$ via the activation of the R-type Ca^{2+} channel and the blockade of this sustained increase by MV8608 (10^{-9}M) in heart cells;

Figure 17 shows the blockade by MV8608 (10^{-9}M) and the absence of the effect of nifedipine (10^{-6}M) on the sustained depolarization (30 mM), and PAF (10^{-9}M) induced sustained increase of $[Ca]_i$ via activation of the R-type Ca^{2+} channel in human heart cells;

Figure 18 represents histograms showing MV8608 (10^{-9}M) blockade of sustained increase of $[Ca]_i$ (in presence of nifedipine (C+N) induced by sustained depolarization (KCl, 30 mM) and PAF (10^{-9}M) stimulation of the R-type Ca^{2+} channel in human heart cells;

Figure 19 represents histograms illustrating MV8608 (10^{-9}M) blockade of the sustained increase of $[Ca]_i$ induced by sustained depolarization (in presence or absence of nifedipine), PAF and ET-1 stimulation of R-type Ca^{2+} channel in chick heart cells;

Figure 20 shows the blockade by MV8608 (10^{-9}M) of bradykinin (10^{-6}M) induced sustained increase of $[Ca]_i$ (in presence of (10^{-6}M) nifedipine) via activation of the R-type Ca^{2+} channels in chick heart cells, human heart cells and rabbit aortic vascular smooth muscle cells;

Figure 21 represents histograms showing the MV8608 (10^{-9}M) blockade of bradykinin (BK,10^{-6}M) and PAF (10^{-9}M) induced sustained

increase of $[Ca]_i$ via activation of the R-type Ca^{2+} channels in rabbit aortic vascular smooth muscle cells;

Figure 22 shows a typical example of the decrease of basal sustained increase of $[Ca]_i$ by MV8608 (10^{-9}M) in freshly isolated human aortic endothelial cells and the blockade by MV8608 (10^{-9}M) of PAF (10^{-9}M) induced sustained increase of $[Ca]_i$ via activation of the R-type Ca^{2+} channels in freshly isolated human aortic vascular smooth muscle cells;

Figure 23 represents histograms showing that increasing the concentration of PAF (10^{-7}M) required high concentration of MV8608 (10^{-6}M) for blockade of PAF induced sustained increase of $[Ca]_i$ via activation of the R-type Ca^{2+} channels in human aortic vascular smooth muscle cell lines;

Figure 24 represents histograms showing that in double-perfused mesenteric bed of the rat, MV8608 (1 µM) but not Illusteol (1 µM) blocked PAF but not ACh and AngII induced arterial vasodilatation and venconstriction;

Figure 25 shows the time course decreases of the TTX-sensitive fast Na^+ current in chick heart cells by (10^{-8}M) of MV8612;

Figure 26 shows the time course blockade of the L-type Ca^{2+} current by high concentration (10^{-7}M) of MV8612 in human heart cells;

Figure 27 shows graphs and cell attached single R-type Ca^{2+} channel recording (in presence of (10^{-6}M) nifedipine) showing the decrease of the single channel current amplitude (panel A, current voltage relationship, n=3), probability of opening (panel B, open probability-voltage relationship, n=3) by 10^{-7}M MV8612 application in the patch pipette and panel C, example of single channel current traces. Panels D-E show that application of MV8612 (10^{-9}M) to extrapipette solution only induced a slight decrease of the R-type Ca^{2+} channel amplitude and largely increased the probability of opening of the channel. This demonstrates that MV8612 does penetrate to the cytosol and its effect at the cytosolic side of the channel is different from that at the outer side;

Figure 28 represents graphs showing that both MV8608 (10^{-8}M) and MV8612 (10^{-8}M) but not nifedipine (10^{-7}M) significantly decreased the spontaneous proliferation of human aortic vascular smooth muscle cell line;

Figure 29 represents histograms showing that the L-type Ca^{2+} blocker, nifedipine did not affect basal cytosolic ($[\ ]_c$) and nuclear ($[\ ]_n$) free Ca^{2+} as well as the sustained depolarization and high PAF induced sustained increase of $[Ca]_c$ and $[Ca]_n$. However, MV8608 and MV8612 blocked completely the sustained depolarization induced sustained increase of $[Ca]_c$ and $[Ca]_n$ (panel A) in heart cells. Panel B shows that high concentration of PAF (10^{-7}M) induced sustained increase of $[Ca]_c$ and $[Ca]_n$ is blocked by high concentration of MV8608 (10^{-6}M) but normal concentration of MV8612 (10^{-8}M);

Figure 30 is a 3-dimensional reconstitution showing the absence of effect of nifedipine and the blockade of the sustained increase of $[Ca]_c$ and $[Ca]_n$ induced by sustained depolarization and high PAF (10^{-7}M) in chick heart (A) and human aortic vascular smooth muscle cell line (B);

Figure 31 represents histograms showing the preventive effect by MV8608 and MV8612 of sustained depolarization and high PAF (10^{-7}M) induced sustained increase of $[Ca]_c$ and $[Ca]_n$ via stimulation of the R-type Ca^{2+} channel in chick heart cells and human aortic vascular smooth muscle cell line;

Figure 32 represents histograms showing the preventive effect by MV8612 (10^{-8}M) of sustained depolarization (KCl 30 mM) and high PAF (10^{-7}M) induced sustained increase of $[Ca]_i$ via activation of the R-type Ca^{2+} channel in human aortic vascular smooth muscle cell line;

Figure 33 represents histograms showing the blockade by MV8612 (10^{-9}M) of sustained depolarization and ET-1 (10^{-9}M) induced sustained increase of $[Ca]_i$ via activation of the R-type Ca^{2+} channels in rabbit aortic vascular smooth muscle;

Figure 34 represents histograms showing the blockade by 10^{-9}M MV8612 of the sustained depolarization, low PAF (10^{-9} M) and ET-1

(10^{-9}M) induced sustained increase of $[Ca]_i$ via the activation of the R-type Ca^{2+} channels in chick heart cells;

Figure 35 shows the marked intrinsic hypotensive properties of the dual L- and R-type Ca^{2+} channel blocker isradipine (panel B) when compared to the pure L-type Ca^{2+} channel blocker, nifedipine (Panel A);

Figure 36 shows that pretreatment with MV8612 abolishes the bronchoconstrictive responses and the hypotensive effect of PAF in the anaesthetized guinea pig model;

Figure 37 shows the effect of subplantar injection of compound MV 8608 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of des-Arg^9-bradykinin (DABK) (A) in rats treated 24 h prior with LPS; bradykinin (BK, B) and for des-Arg^9-bradykinin (C) and for bradykinin (D) in animals treated 30 days prior with BCG. Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 38 shows the effect of subplantar injection of MV 8608 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of prostaglandin E_2 (PGE_2) (A), PAF- acether (PAF) (B), substance P (SP) (C) and ovalbumin (OVO) (D). Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$. Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 39 shows the effect of subplantar injection of MV 8608 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of low dose of des-Arg^9-bradykinin plus PAF (A) or prostaglandin E_2 (PGE_2) (B). Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 40 shows the effect of subplantar injection of MV 8608 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of carrageenan (Cg) (A), dextran (DEX) (B), histamine (HIST) (C) and for

serotonin (5-HT) (D). Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 41 shows the effect of subplantar injection of MV 8612 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of bradykinin (BK) (A and C), des-Arg^9-bradykinin (DABK) (B) and for tyr^8-bradykinin (D). Experiments for DABK were carried out in animals treated with LPS 24 h prior. Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 42 shows the effect of subplantar injection of MV 8612 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of prostaglandin E_2 (PGE_2 (A), PAF acether (PAF) (B), carrageenan (Cg) (C) and substance P (SP) (D). Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$. Each group represents the mean of 4 - 5 animals;

Figure 43 shows the effect of subplantar injection of MV 8612 isolated from *Mandevilla velutina* on paw oedema caused by subplantar injection of low dose of bradykinin plus CGRP (A), BK plus prostaglandin E_2 (PGE_2) (B); BK plus PAF C) or BK plus PGI_2 (D). Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 44 shows the effect of an intraperitoneal injection of MV 8612 isolated from *Mandevilla velutina* on rat paw oedema caused by subplantar injection of bradykinin in animals treated with cyproheptadine (A) or compound 48/80 (B). Each group represents the mean of 4 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 45 shows a time-dependent antioedematogenic effect caused by subplantar injection of compound MV 8612 on bradykinin-induced rat paw oedema. Each group represents the mean of 4-5 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: * $P < 0.05$;

Figure 46 shows a dose-dependent antioedematogenic effect caused by co-injection of compound MV 8612 on bradykinin (BK, A), carrageenan (B), PAF (C and serotonin (D)-induced mouse paw oedema. Each group represents the mean of 7 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels:* $P < 0.05$; ** $P < 0.01$;

Figure 47 shows a dose-dependent antioedematogenic effect caused by intraperitoneal injection of compound MV 8612 on bradykinin (BK, A), cellulose sulphate (B)-serotonin (5-HT, C) and histamine (Hist, D)-induced mouse paw oedema. Each group represents the mean of 5-6 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: ** $P < 0.01$;

Figure 48 shows a dose-dependent antioedematogenic effect caused by intraperitoneal injection of compound MV 8608 on histamine (Hist, A) -serotonin (5-HT, B) and bradykinin (BK, C)-induced mouse paw oedema. Each group represents the mean of 5-6 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: *$P < 0.05$; ** $P < 0.01$;

Figure 49 shows the effect of compound MV 8612 given intraperitoneally on carrageenan (1mg/site)-induced pleurisy in mice. Each group represents the mean of 8 to 10 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: *$P < 0.05$; ** $P < 0.01$;

Figure 50 shows the effect of compound MV 8608 given intraperitoneally on carrageenan (1mg/site)-induced pleurisy in mice. Each group represents the mean of 8 to 10 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: ** $P < 0.01$;

Figure 51 shows the effect of compounds MV 8608 and MV 8612 given intraperitoneally on PAF-acether (1 g/site)-induced pleurisy in mice. Each group represents the mean of 10 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: ** $P < 0.01$;

Figure 52 shows a dose-dependent inhibition of bradykinin-induced skin vascular permeability in rats caused by intraperitoneal

injection of MV 8612 and MV 8608. Each group represents the mean of 8 animals and the vertical bars the S.E.M. The asterisks indicate the significance levels: *P <0.05; ** P < 0.01;

Figure 53 shows a concentration-dependent inhibition of human lymphocyte proliferation caused by compounds MV 8612 and MV 8608. Each group represents the mean of 6-7 experiments and the vertical bars the S.E.M;

Figure 54 shows a dose-related antinociceptive effect caused by intraperitoneal injection of compounds MV 8612 and MV 8608 against acetic-acid-induced writhe responses in mice. Each group represents the mean of 8 animals and the vertical bars indicate the S.E.M;

Figure 55 shows a dose-related antinociceptive effect caused by intraperitoneal injection of compounds MV 8612 and MV 8608 against acetylcholine-induced writhe responses in mice. Each group represents the mean of 8 animals and the vertical bars indicate the S.E.M;

Figure 56 shows a dose-related antinociceptive effect caused by intraperitoneal injection of compounds MV 8612 and MV 8608 against kaolin-induced writhe responses in mice. Each group represents the mean of 8 animals and the vertical bars indicate the S.E.M; and

Figure 57 shows a dose-related antinociceptive effect caused by i.c.v. injections of compounds MV 8612 and morphine against acetic acid-induced writhe responses in mice. Each group represents the mean of 8 animals and the vertical bars indicate the S.E.M.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawing which is exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention thus concerns the saponin-like compounds isolated from *Mandevilla* species with specific R-type calcium channel blocking properties and more particularly to saponin-like compounds from *Mandevilla velutina* and *Mandevilla Illustris* which display the remarkable property of virtually only affecting the overstimulation of R-type Ca^{2+} channels, without significantly affecting the basal activity thereof. In addition, these compounds, and especially MV8608 and MV8612, display the remarkable property of blocking or relieving the side effects associated with other drugs or compounds. Furthermore, the present invention concerns R-type Ca^{2+} channel blockers showing significant activity in a number of applications ranging from a control of cellular proliferation to pain control.

The freshly collected rhizomes of *Mandevilla velutina* were extracted with ethyl acetate and then fractionated by column chromatrography on silica gel with methylene chloride and ethyl acetate as solvents giving 20 components. Two of these fractions showed indirect bradykinin blocking action. As mentioned previously, one of them named Velutinol (MV8608) shows that the structure comprises a pregnane skeleton. The structure of Velutinol A was determined (Yunes et al.,1993, Phythochemistry. 34:787-790; 1993, Phytochemical Analysis 4:76-81; 1993, Phytochem. Anal. 4:76-81; Bento, 1996, J. Chem. Soc. Perkin Trans 2:1359-1366; Yunes et al., 1996) as : 3-ß-hydroxipregna-5-one derivatives (see Figure 1, compounds I, IA and IB) and it was suggested to be a (15R, 16R, 20S)-14, 16:15,20:16,21-triepoxi-15,16-seco 14β, 17α-pregn-5-ene-3β, 15-diol. Figure 1, (compounds I, IA and IB).

Pregnane derivatives have been reported to be present in several species (Abe et al., 1976, Phytochemistry 15:1745-1748; 1978, Chem. Pharm. Bull. 26 (10):3023; 1979, Chem. Pharm. Bull. 27 (7):1604-1610; 1981, Chem. Pharm. Bull. 29 (2):416; 1987, Chem. Pharm. Bull. 35 (10):4087; 1988, Chem. Pharm. Bull. 36 (2):612; 1988, Chem. Pharm. Bull. 36 (10):3811). In any

event, others isomers could also exist as are shown by structures II, III, IV, V and VI (Fig. 1). According to the Calixto group, these isomers have also been shown to have activity through the bradykinin receptor.

For comparison structure of 5-pregnane-3ß-ol-20-one is shown in Figure 1 (compound V).

Surprisingly, it was discovered that compound MV8608 had an inhibitory activity on the R-type calcium channel. Furthermore, this inhibitory activity was shown to be specific to the R-type calcium channel.

Isolated from the same *Mandevilla velutina* rhizomes an other compound MV8612 has a very specific inhibitory activity on the steady-state calcium channel type R. The structure of MV8612 was determined. The invention also relates to the structure of MV8612 (analogs A and B compound, Figure 2) and its saponin-like derivatives displaying an inhibitory activity of the steady-state R-type calcium channel. The primary structure of such compounds is shown in Figure 2.

It is important to note that the molecule of the present invention consists of a classical saponin oligosugar part (designated as "ES" in Figure 2) and a steroid ("T") portion. The structure of the steroid (T) component of the molecule is based on a 5 pregnane-3ß-ol derivative with a tricyclic oxygenated ring system or illustrol isomer as shown in Figure 1 (compounds V and VI). However, as will be recognized by a person of ordinary skill to which the instant invention pertains, derivatives of these compounds can possess inhibitory activity on the Ca^{2+} influx into the cytosol, the nucleus, the mitochondria as well as the (SR) sarcoplasmic reticulum and (ER) endoplasmic reticulum, in the EST combination as shown in Fig. 2. The structure of "T" is preferably a 5-pregnane-3ß-ol oxytricyclo 15-ol as shown in Fig. 2, although a 5-pregnane-3ß-ol-20-one, cholesterol, cholic acid, ergosterol, stigmasterol, androstenon, digitoxygenin, ß-sitostenol, uvaol, ursolic acid, sarsasapogenin, 18, ß-glycyrrhetinic acid, betulin, betulinic acid, oleanoic acid, podocarpic acid are also encompassed as being within the scope of the present invention.

In the EST formula (Figure 2, analog A), S is preferably α(1-4) (2-deoxy, 3-methoxy) -L-lyxotetrose, α(1-4) (2-deoxy, 3-methoxy) L-xylotetrose, α(1-4) (2-deoxy, 3-methoxy)-L-arabinotetrose, α(1-4) (2-deoxy, 3-methoxy)-L-xylotetrose, α(1-4) (2-deoxy, 3-methoxy-L-ribopyranotetrose, α(1-4) (2-deoxy, 3 methoxy-L-sorbotetrose, α(1-4)-L-lyxotetrose, α(1-4)-L-xylotetrose, α(1-4)-L-arabinotetrose, α(1-4)-L-xylotetrose, α(1-4)-3, 4 methoxy-L-lyxotetrose, α(1-4)-3, 4 methoxy-L-xylotetrose, α(1-4)-3,4 methoxy-L-arabinotetrose, α(1-4)-3,4 methoxy-L-xylotetrose, α(1-4)-3,4 methoxy-L-ribopyranotetrose, α(1-4)-3,4 methoxy-L-sorbopyranotetrose, α(1-4)-L-lyxotetrose, α(1-4)-L-xylotetrose, α(1-4)-L-arabinotetrose, (1-4)-L-riboyranoetrose,α(1-4)-L-sorbotetrose.

The MV8612 analog A has a monomeric to oligomeric of mentioned sugar derivatives, and has preferably a tetra sugar derivative. The terminal E of the analog A part is preferably 4-acetoxy-3 methoxy-L-α-lyxose, 4-acetoxy-3-methoxy-L-α-xylose, 4-acetoxy-3-methoxy-L-α-arabinose, 4-acetoxy-3-methoxy-L-α-xylose, 4-acetoxy-3-methoxy-L-α-ribopyranose, 4-acetoxy-3-methoxy-L-α-sor-bose-acetoxy.

The compound of formula I (I, IA and IB) and MV8612 analogs VIIA and VIIB could be modified into peptidic analogs (deprotection reaction of amines functions in peptidic syntheses, acid treatment or catalytic hydrogenation depending on the nature of the ES) in order to obtain peptidic analogs of compounds of formula I as commonly known to a person of ordinary skill. The compounds of formula I (IA and IB) could be, if necessary purified using classical technique such as crystallisation and/or silice column chromatography.

As used herein, "chemical derivatives" is meant to cover additional chemical moieties not normally part of the subject matter of the invention. Such moieties could affect the physico-chemical characteristic of the derivative (i.e. solubility, absorption, half life and the like, decrease of toxicity). Such moieties are examplified in Remington's Pharmaceutical Sciences (1980).

Methods of coupling these chemical-physical moieties to a polypeptide are well known in the art.

As used herein, the terms "molecule", "compound" or "ligand" are used interchangeably and broadly to refer to natural, synthetic or semi-synthetic molecules or compounds. The term "molecule" therefore denotes for example chemicals, macromolecules, cell or tissue extracts (from plants or animals) and the like. Non limiting examples of molecules include nucleic acid molecules, peptides, antibodies, carbohydrates and pharmaceutical agents. The agents can be selected and screened by a variety of means including random screening, rational selection and by rational design using for example protein or ligand modelling methods such as computer modelling. The terms "rationally selected" or "rationally designed" are meant to define compounds which have been chosen based on the configuration of the interaction domains of the present invention. As will be understood by the person of ordinary skill, macromolecules having non-naturally occurring modifications are also within the scope of the term "molecule". For example, peptidomimetics, well known in the pharmaceutical industry and generally referred to as peptide analogs can be generated by modelling as mentioned above. Similarly, in a preferred embodiment, the polypeptides of the present invention are modified to enhance their stability. It should be understood that in most cases this modification should not alter the biological activity of the interaction domain. The molecules identified in accordance with the teachings of the present invention have a therapeutic value in diseases or conditions in which the physiology or homeastasis of the cell and/or tissue is compromised by a defect in Ca^{2+} homeostasis. Alternatively, the molecules identified in accordance with the teachings of the present invention find utility in the development of more efficient compounds to reduce or prevent overstimulation of R-type Ca^{2+} channel associated diseases. As exemplified herein, the present invention provides numerous assay systems to test the effect of these molecules.

They can be also ionized with an acceptable pharmaceutical acid, or if it is possible and if it desired with an acceptable pharmaceutical base.

The necessary crude materials used in the processes described herein are either commercially available or easily accessible to a person of ordinary skill to which the present invention pertains knowledgeable of the instant invention and of the procedures available in the literature.

In comparison with the dual L and R-type Ca^{2+} channel blocker, israpidine (PN200-110), the compounds of the present invention, presents a highly superior *in vivo* as well as *in vitro* specificity and potency as well as protective and therapeutics cellular activities against Ca^{2+} overload in all cell types. Non-limiting examples of such cell types include heart, vascular smooth muscle, vascular and non vascular endothelial cells, bone cells, T lymphocytes, monocytes, smooth muscle cells, nerve cells, cerebral cells, and non-differentiated cells of anaplasic or neoplasic origins.

The tests realized *in vitro* on VSMC, VEC, bone cells, blood immune cells and heart cells in culture, placed in several pathological, electrical and hormonal conditions showed that the compounds of the present invention protected and blocked in a remarkable way and more potently than isradipine, cell integrity and Ca^{2+} overload as well as Ca^{2+}-dependent over stimulation of hormone secretion and abnormal excitation-contraction coupling and conduction. Other tests carried out *in vitro*, using abnormal proliferation of T-lymphocytes as well as VSM, VEC and osteoblast cells, demonstrated that the compounds of the present invention significantly and remarkably protected the cells from proliferation, significantly largely decreased their capacity to undergo spontaneous proliferative processes and retained their normal integrity and function. The effects of the compounds of the present invention were largely superior to that of isradipine.

The tests *in vivo*, using rats and rabbits as well as guinea pigs as model systems for warm blooded animals demonstrated that the compounds of the present invention significantly prevented and blocked vasoconstriction,

hypotension and airways hypereactivity induced by PAF, ET-1 and organ transplantation without any side effect. On note, the activity of the compounds of the present invention was shown to be superior to be largely superior to that of isradipine which was, in addition and in contradistinction to the compounds of the instant invention, the dual L-and R-type channel blockers isradipine, exhibited significant side effects.

The remarkable properties of the compounds of the present invention make them valuable compounds in treatment of numerous diseases and conditions in which R-type Ca^{2+} channel blocking is beneficial. Non-limiting examples of such diseases or conditions include diseases of the cerebral, cardiac and vascular systems, and the immune system, for the treatment and prevention of cerebral and cardiac ischemia, vascular contraction, oedema, post-surgery and post-transplantation hyper-immune activities and related pathologies and septic shock.

In general, the protective effects of the compounds of the present invention and in particular of MV8612 find utility in the treatment of, for example, cardiac, vascular and cerebrovascular accidents of different origin, post-surgical traumas, encephalopathy, neuro-degenerative pathology, hypertrophy, cancer, diabetes type II, hyperthyroidism, osteoporosis, arrythmia, fibrillation as well as osteoporosis.

The property of the compounds of the present invention to protect cells during hypoxia and ischemia as well as remodelling also permits their use in the treatment and prevention of ischemia of peripheral tissues, mainly in cardiology for myocardial ischemia and coronary ischemia and their different clinical expressions such as for example angina, myocardial infarct, arrhythmias, vasospasms, heart failure, fibrillation; as well as in ophthalmology and in oto-rhino-laryngology during chorio-retinial vascular damage, vertigo of vascular origin, vertigo de Meuniere or d'acouphenes as well as digitalis intoxication.

The invention concerns also the addition of salts to the compounds of the present invention and in particular to the compounds of formula I (I, IA and IB) and MV8612 analogs VIIA and VIIB obtained with a mineral or organic pharmaceutically acceptable salt.

The pharmaceutically acceptable acids that can be used to obtain a salt, by addition to the compounds of the present invention, are well known to the person of ordinary skill and taught for example in Remington Pharmaceutical Sciences (1980). Non-limiting examples of pharmaceutically acceptable acids include chlorhydric acid, phosphoric acid, tartaric acid, malic acid, fumaric acid, oxalic acid, methanesulfonic acid, ethanesulfonic acid, camphoric acid, citric acid, etc.

Non-limiting examples of pharmaceutical bases which can saltify the compounds of the present invention and in particular the compounds of formula I (I, IA and Ib) and analogs VIIA and VIIB, include sodium, potassium, calcium, aluminium hydroxyl, carbonates of acaly metals or alkalinoterrus or organic bases such as triethylamine, benzylamine, diethanolamin, tertbutylamin, dicyclohexylamin, arginine, etc.

The present invention also relates to the pharmaceutical compositions including as an active ingredient, a saponin-like compound of the present invention. More particularly, a compound of the present invention having the EST structure as shown in Figure 2 and, even more particularly, a compound of formula I (I, IA and IB) and MV8612 analogs VIIA and VIIB or their salt derivatives (by addition for example of a mineral or organic base or acid) together with a pharmaceutically acceptable carrier, as well known in the art. Non-limiting examples of such pharmaceutically acceptable carriers include inert excipients, non toxic covenants for pharmaceutical use and/or an agents attaching an aromatic agent, a delitement agent, edulcorant agent, lubrificant agent as well as a liquid and semi-liquid vehicle adapted for different modes of administration such as for example sterile epirogenic water for intravenous administration (see for example Remington Pharmaceutical Sciences (1980)).

Non-limiting examples of pharmaceutical compositions according to the invention include, in particular, those adapted for oral, parental, ocular, per or transcutan, nasal, rectal, perlingual administrations such as ocular or nasal drops, pills, sublingus pills, capsules, tablets, suppositories, cremes, pomades, gels, and the like (see for example Remington Pharmaceutical Sciences (1980)).

The compositions of the present invention are generally presented in a dose form and can contain dependent on the patient treated, age and sex of the patient, from 0.1 to 500 mg of the active principle.

It can, depending on the route of administration be delivered at a dose of 0.1 to 500 mg of one or several times a day.

The terminology "pharmaceutical" is used herein in a broad sense to cover veterinary uses. The compositions will be readily adapted by the skilled artisan for the treatment of particular warm blooded animals to which the instant invention pertains.

From the specification and appended claims, the term therapeutic agent should be taken in a broad sense so as to also include a combination of at least two such therapeutic agents. Further, compounds according to the present invention can be introduced into warm blooded animals including human patients in a number of ways, as well known in the art. Erythropoietic cells can be isolated from the afflicted individual, transformed with a DNA construct according to the invention and reintroduced to the afflicted individual in a number of ways, including intravenous injection. Alternatively, the DNA construct can be administered directly to the afflicted individual, for example, by injection in the bone marrow. The DNA construct can also be delivered through a vehicle such as a liposome, which can be designed to be targeted to a specific cell type, and engineered to be administered through different routes.

For administration to humans, the prescribing medical professional will ultimately determine the appropriate form and dosage for a

given patient, and this can be expected to vary according to the chosen therapeutic regimen (i.e. DNA construct, protein, cells), the response and condition of the patient as well as the severity of the disease.

Composition within the scope of the present invention should contain at least one of the active agents in an amount effective to achieve the desired therapeutic effect while avoiding adverse side effects. Typically, the compounds in accordance with the present invention (i.e. MV8608 or MV8612) can be administered to warm blooded animals (i.e. humans) in doses ranging from 0.005 to 1 mg per kg of body weight per day of the warm blooded animal which is treated. Pharmaceutically acceptable preparations and salts of the active agent are within the scope of the present invention and are well known in the art (Remington's Pharmaceutical Science, 16th Ed., Mack Ed.). The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the patient. Typically, 0.001 to 50 mg/kg/day will be administered to the warm blooded animal.

The present invention is illustrated in further detail by the following non-limiting examples.

EXAMPLE 1

Procedure for isolation and purification of compound MV8612 (Figure 2)

The rhizomes of *Mandevilla velutina* were grounded into small pieces and extracted repeteadly with ethyl acetate. The extract was filtered and evaporated to yield a brown powder that accounts for 9% of the rhizomes. The extract was fractioned by silica gel column chromatography with a methylene chloride system containing increasing amounts of ethyl acetate.

Fractions were collected and monitored by thin layer chromatography (TLC; Silica Gel G), eluted with toluene-EtoAc-MeOH (55:45:5) and visualized with short and long wavelength u.v. light or with an arylaldehyde-AcOH-MeOH-H_2SO_4 (0:5:10:85:5) spray.

Fractions rich in velutinol glycoside MV8612 were rechromatographed in the same manner several times. Further purification by TLC yields the pure compound VIIB (Fig. 2) crystallized in ethanol.

Compound VI (0.0001% of dry weight) mp 148-150°C, white needles from ethanol responded positively to the Lieberman Burchard (Abisch et al., 1960, Helv. Chim. Acta 43:1844), Xanthydrol (Barton et al., 1952, Nature 170:249) and Keller-Kiliane (Nagata et al., 1957, Helv. Chim. Acta 40:41) indicating a stereoidal glycoside of a 2-deoxysugar.

The molecular formula was obtained through elementar analysis [((60.26%), H (7.94%), 0 (31.10%)] [Cale:((60.80%) H(8.01%), O(31.10%) and fast atom bombardment (FAB) mass spectrum (MS) that afforded a molecular peak at m/z 1205 ($M + Na^+$); 1221 ($M + K^+$) and 1200 ($M + NH_4^+$) suggesting to be $C_{60}H_{94}O_{23}$. The IR spectrum showed peaks (KBr) at cm^{-1}: 3450 (-OH), 1745 ($-COCH_3$), 2920, 1440 (OCH_3), 1230, 1160, 1100, 1080, 1050 (O-C-O). Its methanolic solution is transparent in the UV visible region. In the mass spectrum the loss of fragment 45 from the aglicone, following by the loss of a fragment of 244, were indicative of a one terminal sugar with two acetyl and one methoxyl groups and suggesting a straight chain of sugars. The positive ionization fast atom bombardment mass spectra (FAB-MS) confirmed the result with peaks at m/z (%) 1137 $[M-Co-OH]^+$ (66) 893 $[1137-C_{11}H_{17}0_6]^+$(10) 749$[893-C_7H_{12}O_3)]^+$ (21), 605 [749-C H O $_3$](25); 462 [605-(H O)$_3$ (10), 318$[462-C_7H_{12}O_3]$+ (15) and suggested that there are four dideoxy sugars in the molecule.

EXAMPLE 2

NMR Experiments

The results of the NMR experiments are shown in Figures 3-6.

1) 1D ^{1}HNMR Spectrum

The analysis of the 600 Mhz ^{1}H NMR spectrum can be divided into three distinct regions. The first region [5.78 ppm-4.28 ppm] with the

best resolved signals, corresponds to the five anomeric protons and protons at C2 and C4 of the sugar ring 5, besides protons 16, 6, 15 (H), 15(OH), 10 and 21b of the genin part (velutinol).

For the second region [3.93 ppm - 3.15 ppm], a very crowded region, the integration is proportional to 31 protons and were assigned as protons 3 and 21a of the genin part, five methoxy and fourteen methine protons of the sugar rings.

The integration of the last region [2.53 - 1.08 ppm] showed the presence of fifty protons; five secondary methyl, eight methylenic, two methyl from acetyl groups and twenty-one protons from the genin part.

2) 1D selective TOCSY

The 1D selective TOCSY was used to define each one of the five spin systems for the sugar rings attached to the genin part. Selective irradiation of an isolated spin multiplet yields a subspectrum of all hydrogens directly or indirectly scalar, coupled to the irradiated resonance, if the mixing time is long enough to allow complet transfer of magnetization. A description of the results is given in Table 1.

TABLE 1

1D selective TOCSY

Irradiated proton	Observed signals (8, ppm)					
	H1	H2	H3	H4	H5	H6
H1,R1 δ=4.45ppm	X	Ax:1.58 Eq:2.08	3.81	3.22	3.85	1.22
H1,R2 δ=4.76ppm	X	Ax:1.64 Eq:2.11	3.78	3.19	3.34	1.21
H2,R3(ax) δ=1.57ppm	4.45	Ax:1.57 Eq:2.29	3.40	3.15	3.28	1.29
H1,R4 δ=4.96ppm	X	Ax:1.55 Eq:2.16	3.77	3.19	3.92	1.21
H2,R5 δ=5.12ppm	4.43	5.12	3.33	5.33	3.70	1.21

The four anomeric protons of 2,6-dideoxyhexopyranose appeared as a doublet of doublets at 4.96; 4.85; 4.76 and 4.45, with J = 10 and 2 Hz. A fifth anomeric proton appeared as a doublet (J = 10 Hz), 4.43 and was assigned to the normal hexapyranose unit. The large value of the coupling constant of these anomeric protons were typical of the axial configuration of the hexopyranoses in the C-1 (D) conformation indicating that these sugars were joined through (1 → 4)-glycosidic linkages.

The spectrum also contained five methoxy groups which appear as singlets and were observed at 3.37(3H), 3.44(3H), 3.39(3H), and 3.34(6H); five secondary methyl groups appear as doublets and were observed at 1.21(9H), 1.22(3H) and 1.29(3H), (J = 6.0 Hz); two tertiary methyl groups

singlets were observed at 1.08 and 1.11 and two methyl from acetyl groups were observed at 2.07 and 2.18.

The eight C-2 methylene protons of four 2-deoxy sugar units appeared as two sets of four protons multiplets in the regions 2.29-2.08 and 1.64-1.55 for the equatorial and axial protons respectively (Abe et al., 1988, Chem. Pharm. Bull. 36 (2):612; Abe et al., 1987, Chem. Pharm. Bull. 36 (10):3382-3389). There is also a doublet of doublets at 5.12 (J = 10 and 8 Hz) attributed to the C-2 proton of the acetylated sugar, it couples with both the signals at 4.43 (anomeric proton) and 3.33 credited to a C-3 proton. The C-3 signal, part of a multiplet, is coupled with a doublet of doublets at 5.33 (J=3.0 and 2.0 Hz) attributed to the C-4 proton of a diacetyl sugar, which in turn is coupled with a doublet of doublets at 3.70 (J=6.0 and 2.0 Hz) attributed to the C-5 proton. This is in turn coupled to a doublet (J=6.0 Hz) at 1.21, attributed to the secondary methyl. The chemical shift for C-2 and C-4 is in accordance with an acetyl sugar derivatives.

3c) COSY spectrum

The J coupling relationship described above was also determined from the COSY spectrum. The complete ^{1}H NMR assignment of all the protons in the five sugar rings is given below:

a) H1, 4.43 H2, 5.12 H3, 3.33 H4, 5.33 H5, 3.70 CH3, 1.21.

b) H1, 4.96 H2, 1.55 and 2.16 H3, 3.77 H4, 3.19, H5, 3.92 CH3, 1.21.

c) H1, 4.45 H2, 1.57 and 2.29 H3, 3.40 H4, 3.15 H5, 3.28 CH3, 1.29

d) H1, 4.76 H2, 1.64 and 2.11 H3, 3.78 H4, 3.19 H5, 3.34 CH3, 1.21.

e) H1, 4.85 H2, 1.58 and 2.08 H3, 3.81 H4, 3.22 H5, 3.85 CH3, 1.22.

4) ^{13}C spectrum, DEPTs and CH correlations

The carbon 13 nuclear magnetic resonance spectra (^{13}C NMR) indicated the presence of six quaternary carbons, fourteen methyl, eleven methylene, twenty nine methine and two carbonyl groups (Breitmaier et al., 1987, Third Edition VCH Veriagsgprollachaft mbH weinheim RFG, Germany). The carbon signal were assigned on the ^{1}H-^{13}C COSY (correlated spectroscopy) one-bond spectrum except for the quaternary ones. The long-range correlations data was used to assign these and the multiplicity of the protonated one was determined from the DEPT (distortion enhancement by polarization transfer) spectral data. The ^{13}C assignments of the sugars are indicated in Table 2.

TABLE 2

^{13}C assignments (sugars) (δ, ppm)

	C1	C2	C3	C4	C5	-CH3	-Omc
R1	96.11	35.63	68.38	82.59	70.87	18.25*	56.74
R2	99.73	36.15	77.10	83.91	71.19	18.27*	58.33
R3	101.45	36.35	78.75	82.15	71.54	18.41	56.39
R4	98.47	35.37	76.39	83.81	69.29	18.06	58.03
R5	102.55	70.72	80.34	68.45	71.02	16.58	57.81

(*) These assignments can be interchangeable

CH3-CO			
CH3		CO	
20.87**	C2-R5	170.6	C4-R5
20.97**	C4-R5	172.4	C4-R5

(**) These assignments can be interchangeable

In agreement with the previous determination (ref. of velutinol) of the genin part (Velutinol A), the proton nuclear magnetic resonance spectra (^{1}H NMR) showed a characteristic signal due to the C-6 olefinic proton (5.38, m), as well as those due to C-19 and C-18 (1.08 and 1.11, s) methyl protons, C-3 methine proton (3.53, t,t), C-9, C-8, C-17, C-20, C-15 and C-16 methine protons (1.36, m; 2.01, td; 2.53, d,d; 4.44, d; 5.01, d; and 5.78,d, respectively) and C-21 methylene protons (, 3.81 and 4.28, d) (Abe et al., 1988, Chem. Pharm. Bull. 35 (10):4081-4087; Chen et al., 1987, Phytochem. 26 (8):2351) (Tables 3 and 4).

5) CH long range correlations

The analysis of the ^{1}H - ^{13}C long-range data gave the following correlations which provide evidence for an attachment of the genin and the sugar residue, as well as for the following sequence of the sugar rings: (Table 5)

a) H3(3.35, V) C1(96.11, R1) and H1(4.85, R1) C3(77.64, V) define the linkage point between the genin and the sugar residue;

b) H4(3.22, R1) C1(99.73, R2) and H1(4.76, R2) C4(82.59, R1) define the connection between the first and second sugar rings;

c) H4(3.19, R2) C1(101.45, R3) and H1(4.45, R3) C4(83.91, R2) define the connection between sugar rings 2 and 3;

d) H4(3.15, R3) C1(98.47, R4) and H1(4.96, R4) C4(82.15, R3) define the attachment of sugar ring 3 to sugar ring 4;

e) H4(3.19, R4) C1(102.55, R5) and H1(4.43, R5) C4(83.81, R4) define the linkage point between the last two sugar rings 4 and 5.

TABLE 3

^{13}C assignments for the genin part (velutinol) in compound -12 (δ, ppm)

Position	Velutinol*	Compound-12
1	37.4	37.57
2	31.4	29.67
3	71.3	77.64
4	42.0	38.89
5	139.3	140.49
6	121.4	121.56
7	25.9	26.07
8	33.5	33.69
9	45.8	45.94
10	37.6	37.96
11	18.6	18.68
12	26.6	26.80
13	43.5	43.70
14	87.1	87.40
15	92.5	92.67
16	108.7	108.84
17	52.3	52.47
18	21.6	21.74
19	19.1	19.17
20	73.8	73.96
21	78.0	78.19

* **Breitmaier, E. et al.**, 1987, Carbon 13 NMR Spectroscopy-High Resolution Methods and Applications in Organic Chemistry and Biochemistry, Third Edition VCH Veriagsgprollachaft mbH weinheim RFG, Germany.

TABLE 4

^{1}H NMR assignments of Velutinol and of the aglycone of 8612 compound

Position	ppm δ.-^{1}H	
	Velutinol	Compound 12
1	1.12(a): 1.82(b)	1.14(a)qd: 1.82(b)dt.J=13.5:3.5
2	1.85(a): 1.50(b)	1.95(a): 1.56(b)
3	3.53(H.a): 1.80(OH)	3.53(tt.J=11.5:4.5)
4	2.30(a): 2.23(b)	2.34(a): 2.23(b)
5	--	--
6	5.38	5.38(m)
7	2.16(a): 1.89(b)	2.16(a): 1.90(qd.J=18.0:5.0:2.5)
8	2.01	2.01(td.J=11.5:5.5)
9	1.36	1.36(dd)
10	--	--
11	1.64(a.b)	1.64(a.b)m
12	2.35(a): 1.65(b)	2.35(a): 1.65(b)
13	--	--
14	--	--
15	5.01(H): 4.75(OH)	5.01(H)d.j=12.0: 4.75(OH)d.J=12
16	5.78	5.78(d.J=4.5)
17	2.53	2.53(dd.J=6.0:4.5)
18	1.11	1.11(s)
19	1.09	1.08(s)
20	4.45	4.44(dd.J=6.0:3.5)
21	3.81(A) 4.28(B)	3.81(A)dd.J=10.0:3.5 4.28(B)d.J=10.0

TABLE 5

^{13}C-^{1}H correlation long range

C-1 (δ, ppm)		H (δ, ppm)	
R-1	96.11	3.53	H3(Vel.)
R-2	99.73	3.22	H4-R1
R-3	101.45	3.19	H4-R2
R-4	98.47	3.15	H4-R3
R-5	102.55	3.19	H4-R4

C (δ, ppm)		H-1 (δ, ppm)	
C-3 (velutinol)	77.64	4.85	R-1
C4-R1	82.59	4.76	R-2
C4-R2	83.91	4.45	R-3
C4-R3	82.15	4.96	R-4
C4-R4	83.81	4.43	R-5

6) NOESY spectra

The analysis of the cross peaks in the NOESY (2D n.O.e) spectra also provides evidence for the above mentioned connections between the sugar rings and the genin. The most important interactions are the following:

H1(4.43, R5) H4(3.19, R4); H1(4.96, R4) H4 (3.15, R3);
H1(4.45, R3) H4(3.19, R2); H1(4.76, R2) H4(3.22, R1);
H1(4.85, R1) H3(3.53, V); H1(4.85, R1) H4 (2.34, V).

The analysis of the NOESY spectra also showed that in each of the sugar rings, proton at C-3 couple with proton in the C1, and this is in turn spatially coupled to proton at C-5 position. Such proximities can occur if these protons are all axial. This thus provides evidence that the methoxy and the secondary methyl groups are located at equatorial positions.

The values for the coupling constants **(J)** for protons 2 (10 and 8 Hz) and 4 (3.0 and 2.0 Hz) of the last sugar ring (5.12 and 5.33, respectively) indicate their position as axial configuration at C-2 and an equatorial configuration at C-4. The acetoxy groups are at equatorial and axial positions, respectively. In addition, the long range CH correlation spectra showed coupling between the two carbonyl at 170.6 ppm (acetyl at C-2) and 172.4 ppm (acetyl at C4), with the methyl signals at 2.08 ppm and 2.17 ppm, respectively.

The analysis of NMR spectra, specially long range CH correlations and cross peaks in the NOESY spectra, provides information for the connection between the sugar rings and the genin. The data from NMR confirm the sequence of sugars indicated by FAB-MS with the 6-deoxy-2,4-acetoxy-3-O-methyl hexopyranose as the terminal of the sugar chain.

EXAMPLE 3

Structure of the compounds

In the formula **EST** for analog VIIB (Fig. 2) the structure of the **T** part of the molecule is a 5-pregnane-3 -ol-20-one, cholesterol, cholic acid, ergosterol, stigmasterol, androstenon, digitoxygenin, -sitosterol, uvaol, ursolic acid, sarsasapogenin, 18, -glycylrhetinic acid, betulin, betulinic acid, oleanoic acid, padocarpic acid, and preferably 5-pregnane-3 -ol oxytricyclo 15-ol as shown in Fig. 1 (I, IA, IB, II, III, IV).

S for analog B is preferably (1-4) (2-deoxy, 3-methoxy, 5-methyl) -L-lyxotetrose, (1-4) (2-deoxy, 3-methoxy) L-xylotetrose, (1-4)

(2-deoxy, 3-methoxy)-L-arabinotetrose, (1-4) (2-deoxy, 3-methoxy)-l-xylotetrose, (1-4) (2-deoxy, 3-methoxy-L-ribopyranotetrose, (1-4) (2-deoxy, 3 methoxy-L-sorbotetrose, (1-4)-L-lyxotetrose, (1-4)-L-xylote-trose, (1-4)-L-arabinotetrose, (1-4)-L-xylotetrose, (1-4)-3,4 methoxy-L- lyxotetrose, (1-4)-3,4 methoxy-L-xylotetrose, (1-4)-3,4 methoxy-L- arabinotetrose, (1-4)-3,4 methoxy-L-xylotetrose, (1-4)-3,4 methoxy-L- ribopyranotetrose, (1-4)-3,4 methoxy-L-sorbopyranotetrose, (1-4)-L- lyxotetrose, (1-4)-L-xylotetrose, (1-4)-L-arabinotetrose, (1-4)-L- sorbotetrose.

E for analog VIIB is preferably diacetylfucose but also 4-acetoxy-3 methoxy-L-lyxose, 4-acetoxy-3-methoxyl-L-xylose, 4-acetoxy-3-methoxylL-arabinose, 4-acetoxy-3-methoxy-L-xylose, 4-acetoxy-3-methoxy-L-ribopyranose, 4-acetoxy-3-methoxy-L-sorbose-acetoxy.

MV-8608 - Has only one sugar bonded to the genin part T (Fig. 2).

MV-8609 - Has in the part S of Fig. 2 only one sugar.

MV-8210 - Has on the part S of Fig. 2 two sugars.

MV-8611 - Has in the part S of Fig. 2 three sugars.

All of these compounds were demonstrated to be active against bradykinin-induced pharmacological effects (Calixto, Yunes, et al., 1987, *supra*; Calixto et al., 1988, Br. J.Pharmacol., 94:1133-1142).

EXAMPLE 4

Compounds from *Mandevilla illustris*

MI-07 (Illustrol)

The structure of illustrol (Fig. 1 compound VI) was determined by the same authors to be a derivative of 14:15-seco-15-norpregnane (Yunes et al., 1993, *supra*).

MI-15, MI-18 and MI-21

Demonstrated to be active against bradykinin-induced pharmacological effects (Calixto et al., 1991, General Pharmacol. 22:99-101;

1991, Memórias do Instituto Oswaldo Cruz, 86:195-202) are of similar structure to that indicated in Fig. 2, where the Genin (part T) correspond to the illustrol. The S part has one, two three or more sugars and the terminal sugar E is, as was indicated preferably, 6-deoxy-2,4-acetoxy-3-0-methyl hexopyranose.

EXAMPLE 5

Experimental procedures

The freshly collected rhizomes of the *Mandevilla* species were cut into small pieces and repeatedly extracted with ethyl acetate at room temperature. The extract was filtered and evaporated under reduced pressure and the crude extract was fractioned by column chromatography on silica gel using methylene chloride with increasing amounts of ethyl acetate as eluent.

After repeated column chromatography of the fractions using hexane-acetone as eluents, it is possible to isolate several compounds that exhibit indirect bradykinin blocking action.

HPLC chromatogram of MV8608 and MV8612 are observed in Figs.7 and 8 and a CG chromatogram of MV8608 in Fig. 9. The CG chromatogram of Illustrol is shown in Fig. 10.

M.ps. (melting point) were determined using a Kofler-stage microscope and are not corrected. Optical rotation were recorded at 18-28° C using a 1 dm cell. IR spectrum was obtained from KBr discs and in $CHCl_3$ solution. Electron-impact mass spectra were taken on an MS Finnigam model 1020. The FAB-MS were taken on a Fison.

The NMR experiments were made in $CDCl_3$ solution with TMS as internal standard, using AM 250 and Amx 600 instruments, and a JEOL alpha 500 instrument. One-dimensional ^1H spectram were acquired as 64 K data points with a spectral width of 8.5 ppm (0-8.5). One-dimensional ^{13}C spectrum were recorded as 64 K data points with a spectral width of 200 ppm (0-200). The ^{13}C DEPT (distortionless enhancement by polorization transfer) experiments (90 and 135) were both recorded on the Bruker AM 250 spectrometer at 62.89 MHz.

The 1D selective TOCSY were recorded on the JEOL alpha 500 MHz spectrometer. The phase sensitive DQF-COSY and NOESY (nucleus overhouser effect spectroscopy) were acquired using standard Bruker programs. The hereronuclear (^1H - ^{13}C) correlation experiments, both one-bond and long-range correlation, were performed in reverse ^1H detected mode.

TLC was performed on silica gel (Merck Kilselgel 60 F254 0.25 mm layers). Column Chromatography was carried out on silica gel 200-300 mesh).

The plant material of *M.velutina* was collected from Minas Gerais State, Brazil, and was identified by Prof. Ademir Reis and Valério F. Ferreira of the Department of Botany of the Federal University of Santa Catarina. A voucher specimen is deposited in the Herbarium "Flor" of the Department of Botany, Federal University of Santa Catarina, under acession number 17.888-17.892.

EXAMPLE 6

Pharmacological study of the compounds of the present invention:

Principle of *in vitro* studies

Single cells from heart, VSM and VEC of human and animals in culture constitute a model of choice for looking at the effect of drugs on different types of ionic channels using whole-cell and single channel patch clamp techniques in normal and stimulated conditions (Bkaily et al., 1988, *supra*; 1991, *supra*; 1992, *supra*; 1992a, *supra*; 1993a, *supra*; 1996a-e, *supra*; Bkaily G. 1994a,b, *supra*). Indeed, it is recognized as a model system in analyzing drug ionic channel interactions.

Heart cells as well as VSMC possess fast Na^+ current, T, L and R-type Ca^{2+} channels as well as different types of K^+ channels (Bkaily G., 1991, *supra*; 1995, *supra*). However, vascular endothelial cells (VECs) only possess R-type Ca^{2+} channels and different types of K^+ channels. Thus, the later type of VECs constitute a model of choice for studying the effect of drugs on

Ca^{2+} influx due to opening of the R-type Ca^{2+} channels (Bkaily G., 1994, *supra*; 1996, *supra*; 1997a-d, *supra*). Of note, the R-type Ca^{2+} channel has been reported to be responsible for the sustained increase of intracellular calcium and nuclear and cytosolic Ca^{2+} overload that are a result of sustained depolarization of the cell membrane or continual presence of several cardioactive and vasoactive hormones such as ET-1, PAF, bradykinin and insulin (Bkaily G., 1994, *supra*; 1992, *supra*; 1993, *supra*; 1995, *supra*; 1996, *supra*; 1997a, *supra*; 1997b, *supra*; 1997c, *supra*; 1997d, *supra*; 1998, *supra*).

A sustained increase of cytosolic, nuclear and mitochondrial Ca^{2+}, considered as pathological, is a visible and measurable aggression in all types of excitable and non excitable cells such as heart cells, VSMC, VEC, osteoblast cells and immune cells (Bkaily et al., 1996, *supra*).

Herein the effect of the compounds of the present invention were tested on the above-mentioned cell types at whole-cell and cell attached patch clamp configurations, as well as at $[Ca]_i$, $[Ca]_c$ and $[Ca]_n$ levels using a standard techniques (Bkaily, 1994a,b, *supra*; 1992, *supra*; 1993, *supra*; 1995, *supra*; 1996, *supra*; 1997a,b,c,d, *supra*; 1998, *supra*).

Methodology

Single cells of different types in culture were prepared from biopsies of human, chick and rabbit. Known and accepted methods for the isolation of fast Na^+ current, T, L and R-type Ca^{2+} channels as well as delayed outward K^+ current were used (Bkaily 1994, *supra*).

The compounds to be tested are added to the appropriate extracellular solution after recording a stable ionic current or normal steady-state level of $[Ca]_n$ and $[Ca]_n$. The effect of different concentrations of compounds are tested on the different type of current and $[Ca]_i$ of the different cell types. The effect of each concentration of the compounds in function of the time of exposure are then determined. Once the steady-state effect is reached, the second concentration is added, etc.

Also, for the R-type Ca^{2+} channel current, the effect of the compounds are tested on the R-type Ca^{2+} channel amplitude, voltage dependency and probability of opening, by using the cell-attached patch clamp technique (Bkaily 1994, *supra*; 1996, *supra*) and intra and extra patch pipette application of the drug. In all experiment using single channel recording, nifedipine (10^{-6}M) was present in the control and experimental solutions.

Recent results have recently demonstrated that some cardiogenic and vasoconstrictor hormones such as PAF, ET-1 and bradykinin induced a sustained increase of cytosolic as well as nuclear calcium (data not shown). This sustained increase of Ca^{2+} induced by depolarization of the cell membrane or hormones such as PAF, ET-1 and bradykinin is due to the increase of Ca^{2+} influx through the R-type Ca^{2+} channels at the sarcolemmal membrane and/or the nuclear membrane (Bkaily G., 1994, *supra*; 1996, *supra*; 1997a-d, *supra*). Using Ca^{2+} fluorescence probes Fura-2 or Fluo-3 and 2 and three-dimension Ca^{2+} imaging techniques (Bkaily G., 1994, *supra*; 1996, *supra*; 1997a-d, *supra*), the effect of hormones and drugs could be easily tested. These two methods are used with single cells of different types as described above.

The effects of the compounds of the present invention on cytosolic and nuclear Ca^{2+} in different conditions (K^+ depolarization, PAF, ET-1, etc.) that increase the probability of opening of the R-type Ca^{2+} channels and induce cytosolic and/or nuclear Ca^{2+} overload (in presence of L-type Ca^{2+} blocker, nifedipine) were tested.

Using Fura-2 or Fluo-3 cytosolic and nuclear Ca^{2+} measurement techniques, the inventors also tested the effect of the compounds of the invention on the spontaneous increase of cytosolic and nuclear Ca^{2+} during spontaneous contraction of ventricular single cells. Single cells from human fetal ventricular cells and chick embryonic cells were bathed in normal Tyrode's solution and spontaneous intracellular Ca^{2+} transient recorded in the absence and the presence of the compounds of the invention.

EXAMPLE 7

Effects of MV8608 on TTX-sensitive fast Na^+ current

In one series of experiments (n=5), the effect of different concentrations of MV8608 (10^{-11}M to 10^{-7}M) on the TTX-sensitive fast Na^+ current were tested using the whole-cell voltage clamp technique and experimental conditions reported elsewhere (Bkaily et al., 1988, *supra*; 1993, *supra*). MV8608 was found to have no effect on the TTX-sensitive fast Na^+ current at all concentrations used and Figure 11 shows an example using a concentration of 10^{-7}M.

Thus, MV8608 had no effect on the fast Na^+ current and cannot be used as a depressor or blocker of this channel where its reduction has a therapeutic action such as the case of several local anesthetics and antiarrythmic drugs such as Lidocaine.

EXAMPLE 8

Effect of MV8608 on T-type Ca^{2+} current

In another series of experiments (n=7), the effect of different concentrations of MV8608 were tested on the T-type Ca^{2+} current (I_{Ca}) using the whole-cell voltage clamp technique and classical experimental conditions described elsewhere by the inventors (Bkaily G. et al., 1991, *supra*; 1992, *supra*; 1993, *supra*). MV8608 had no effect on the T-type I_{Ca} amplitude at a concentration of 10^{-9}M to 10^{-7}M and Figure 12 shows an example. As it can be seen in that figure, the inset effect of MV8608 in T-type I_{Ca} is immediate. Thus, the MV8608 was found to be a very weak depressor of the T-type I_{ca}.

These results suggest that MV8608 cannot be used as a potent blocker of the T-type Ca^{2+} channel and in a therapeutic action, wherein its blockade is involved. However, the depressing effect of MV8608 on the T-type I_{Ca} could be useful for example, in combination with other drugs to suppress ventricular tachycardia and fibrillation.

EXAMPLE 9

Effect of MV8608 on L-type Ca^{2+} channel

In another series of experiments (n=5), the effect of different concentrations of MV8608 (10^{-11} to 10^{-6}M) were tested on the L-type I_{Ca} of heart cells of chick embryos using the whole-cell and experimental conditions and protocols described elsewhere by the inventors (Bkaily G. et al., 1993, *supra*). As for the T-type I_{Ca}, the L-type I_{Ca} was not affected by 10^{-11} to 10^{-10}M MV8608. However, increasing the concentration of the compound up to 10^{-9}M decreased the I_{Ca} amplitude by 10% and a further slight increase was found at a concentration of 5 x 10^{-7}M of MV8608. Figure 13 shows a typical experiment of the time course effect of the 10^{-9} and 5 x 10^{-7}M concentrations of MV8608.

These results show that MV8608 is a very weak depressor of the L-type Ca^{2+} channel. Thus MV8608 cannot be considered as a high potent antagonist of the L-type Ca^{2+} channel but its depressor effect could be useful when used in combination with known L-type Ca^{2+} antagonist drugs. The weak depressor effect of MV8608 on the T-type Ca^{2+} channel along with the L-type Ca^{2+} channel would be highly beneficial for the treatment of ventricular tachycardia, fibrillation and pathology, where the L-type Ca^{2+} blockers are known and clinicaly used.

EXAMPLE 10

Effect of MV8608 on R-type Ca^{2+} channel

In another series of experiments (n=7), using the cell attached patch clamp technique (Bkaily G. 1994, *supra*; Bkaily G. et al., 1996, *supra*), the effect of 10^{-7}M of MV8608 on the R-type Ca^{2+} channel in human aortic vascular smooth muscle cell line was tested. In one series of experiments (n=4), in the presence of 10^{-6}M of nifedipine (10^{-6}M) in the patch pipette solution containing 110mM Ca^{2+}, 10^{-7}M of MV8608 was applied to the pipette. MV8608 applied in the patch pipette solution decreased the single channel amplitude and probability of opening of the single R-type Ca^{2+} channel under the patch pipette

without affecting its single channel conductance. In a second series of experiments (n=3), the patch pipette solution was free of MV8608 and after recording the single channel activities at different voltages, MV8608 (final concentration of 10^{-7}M) was applied to the extra-patch pipette solution containing 140mM KCl (where the rest of the cell is bathing). This experiment was designed to verify whether MV8608 crossed the cell membrane and if so, whether its effect from the internal side of the channel under the patch is the same as that when applied at the outer side thereof. The results showed that MV8608 did indeed cross the cell membrane. However, instead of decreasing permanently the amplitude and probability of opening of the single R-type channel as did the intra-patch pipette application, the action of MV8608 on the inner side of the membrane increased the probability and duration of opening of the R-type channel. This was accompanied by a spontaneous decrease and release of the blockade of the single channel current. Figure 14 shows a typical single R-type Ca^{2+} channel current in absence and presence of extra-patch pipette 10^{-7}M of MV8608. Such a pattern of increase of probability of opening and the open duration accompanied with the sporadic decrease of the single channel current amplitude has never been observed when MV8608 was applied at the outer side of the single channel under the patch pipette.

These results highly suggest that the blocking action of MV8608 on R-type Ca^{2+} channel is located mainly at the outer side of the channel. Furthermore, it strongly suggests that its capability of crossing the cell membrane enables the compound to increase fleckering and duration of opening of the channel which in turn makes the external inhibitory site of the channel accessible to the external molecules of MV8608. Taken together, these results highly suggest that the inhibitory action of MV8608 requires the R-type Ca^{2+} channel to be in the open state (overstimulated) and also depends on the frequency of opening of the R-type Ca^{2+} channel. This may explain, at least in part, the preventive as well as therapeutic action of MV8608, on the

overstimulation of the R-type Ca^{2+} channel as will be shown below using Fura-2 and Fluo-3 Ca^{2+} measurement techniques.

These results demonstrate that MV8608 does block efficiently the R-type Ca^{2+} at the open state of the channel (i.e. in state of overstimulation). The blockade of the R-type Ca^{2+} by MV8608 should reduce the sustained Ca^{2+} overload that occurred during many abnormal cell function such as for example sustained vasoconstriction and hormone secretion, self-perpetuating hormone secretion of spontaneously active proliferating cells in atherosclerosis, cancer cells proliferation, acute immuno-reaction, arthritis inflammation, pain, ischemia-reperfusion, asthma, acute bronchoconstruction, arrythmia, fibrillation, septic shock and epiptosis.

EXAMPLE 11

Effect of MV8608 on R-type Ca^{2+} channel under sustained activation thereof

In another series of experiments we tested the effect of MV8608 (10^{-9}M) on R-type Ca^{2+} channels stimulation-induced sustained increase of total intracellular Ca^{2+} by sustained depolarization (Figures 15 to 19), by PAF (10^{-9}M) (Figures 16 to 19 and 21 to 22), by ET-1 (10^{-9}M) (Figures 16 and 19) and bradykinin (BK, 10^{-6}M) (Figures 20 and 21), in embryonic chick heart cells (Figures 15, 16, 19 and 20), 19-week-old human fetal heart cells (Figures 15,17,18 and 20), rabbit aortic vascular smooth muscle (VSM) cells (Figures 20 and 21), human aortic VSM cell-line (Figure 23) and freshly isolated (Figure 22) as well as in freshly isolated aortic endothelial cells (Figure 22).

As can be seen in these results, sustained activation of the R-type Ca^{2+} channel induced by a sustained increase of $[Ca]_i$ induced by a sustained depolarization or sustained superfusion with a relatively low concentration of a hormone such as ET-1 (10^{-9}M), PAF (10^{-9}M) and high concentration BK (10^{-6}M), was completely blocked by 10^{-9}M of MV8608 and this effect occurred within 4 to 5 min in the presence of the R-type Ca^{2+} blocker. In

addition, these results showed that the pure L-type blocker, nifedipine (10^{-7}M to 10^{-5}M) had no effect on the R-type Ca^{2+} channel or on the sustained increase of $[Ca]_i$ - induced stimulation of the R-type Ca^{2+} channel (Figures 16 to 23). Furthermore, the pure L-type Ca^{2+} channel blocker did not prevent MV8608 from blocking the stimulation of R-type Ca^{2+} channel induced sustained increase of $[Ca]_i$ (Figures 18 and 19). In some experiments, the stimulation of the R-type Ca^{2+} channel was elevated by increasing the concentration of PAF from 10^{-9}M up to 10^{-7}. Under such conditions, 10 M^9 of MV8608 failed to significantly decrease the sustained increase of $[Ca]_i$ induced by 10^{-7}M PAF. Only a concentration of 10^{-6}M of MV8608 was able to block the high PAF effect on the sustained increase of $[Ca]_i$ (Figure 23).

Taken together, these results demonstrate that MV8608 blocked the R-type Ca^{2+} channel in all cell types used including the human osteoblast cancer cell lines (MG63 and FAOS-2), human VSM cells isolated from atherosclerotic patients, arterial and venous endothelial cells, endocardiac endothelial cells, T-lymphocytes and platelets (not shown) and spontaneously proliferative human aortic vascular smooth muscle cells, AOSMC-9 (Figure 28).

In another series of experiments confocal microscopy was used with Fluo-3, 3-dimension Ca^{2+} measurement techniques in order to verify if the blockade of the R-type Ca^{2+} channel by MV8608 could block both the sustained increase of cytosolic ($[\ ]_c$) and (nuclear ($[\ ]_n$) free Ca^{2+} induced by the stimulation of the R-type Ca^{2+} channels induced by a sustained increase of $[Ca]_i$. As can be seen in Figures 29 and 30, and as previously reported (Bkaily et al., 1996a, *supra*), in presence of nifedipine, sustained depolarization with 30mM KCl and PAF induced a sustained increase of both $[Ca]_c$ and $[Ca]_n$ (largely nuclear). The MV8608 blocked both the cytosolic and nuclear Ca^{2+} sustained overload with a concentration of 10^{-7}M. As was shown using the Fura-2 total $[Ca]_i$ measurement technique, only at 10^{-6}M did the compound significantly decrease the sustained increase of $[Ca]_c$ and $[Ca]_n$ induced with high concentration of PAF (10^{-7}M) back to the control level (Figure 29B). In addition

to its blocking of the depolarization induced sustained increase of $[Ca]_i$, MV8608, at a concentration of 10^{-8} M and 10^{-7} M, prevented the stimulation of the R-type Ca^{2+} channel by the sustained depolarization in a dose-dependent fashion, thus decreasing back the $[Ca]_c$ and $[Ca]_n$ to the control level (Figure 29A). Extracellular applications of the Ca^{2+} chelator EGTA further decreased the $[Ca]_i$ mainly at the nucleus level.

These results demonstrate that blockade of the R-type Ca^{2+} by MV8608 blocked both $[Ca]_c$ and $[Ca]_n$ sustained overload. In contradistinction to what was shown for high PAF (10^{-7} M) induced sustained increase of $[Ca]_i$, $[Ca]_c$ and $[Ca]_n$, MV8608 succeeded in preventing the high PAF action at a concentration of 10^{-9}M. Thus, this compound seems to be equally effective in acute sustained Ca^{2+} overload, however, it is more effective as a preventive blocker of the R-type Ca^{2+} channel in chronic overstimulation of the channel.

Taken together, these results showed that MV8608 is a more effective R-type Ca^{2+} channel blocker in acute rather than in chronic situations. However, it seems to be more effective in preventing rather than treating a chronic stimulation of Ca^{2+} influx through the R-type Ca^{2+} channel. Non-limiting examples of acute situations include septic shock, acute asthma attacks and bronchospasm. Non-limiting examples of chronic situations include cystic fibrosis, rheumatoid arthritis, pulmonary oedema and hypertension caused by arteriosclerosis.

In another series of experiments using the double-perfused bed of the rat (Claing A. et al., 1994, *supra*), MV8608 was found to induce a concentration-dependent inhibition of the PAF-induced responses (Figure 24). The inhibitory properties of MV8608 are specific for PAF as the response to ACh (arterial) and AngII (venous) are unaffected by the pregnane-containing moiety.

In summary, the potency of blockade of the R-type Ca^{2+} channel by MV8608 depends on the degree of the sustained Ca^{2+} overload at the cytosolic and nuclear Ca^{2+}, thus it depends on the degree of overstimulation of the R-type Ca^{2+} channel. Consequently, a blockade of the R-type Ca^{2+}

channel by MV8608 will be beneficial in pathological situation where sustained cytosolic and nuclear Ca^{2+} take place (such as those described above).

EXAMPLE 12

Effect of MV8612 on TTX-sensitive fast Na^{2+} current

In a series of experiments (n=5), we tested the effect of another MV compound MV8612 on the TTX-sensitive fast Na^+ current (I_{Na}) of embryonic chick heart cells using the whole-cell voltage clamp technique described above. Superfusion with 10^{-9}M of MV8612 had no effect on the fast Na^+ current. However, increasing the concentration up to 10^{-8}M decreased the I_{Na} amplitude by 15% and no further decrease was found at 10^{-7}M. Figure 26 shows a typical example of the time course effect of 10^{-8}M MV8612 on the peak amplitude of the fast I_{Na}.

These results show that unlike MV8608, MV8612 depresses the fast Na^+ current in heart cells. This compound could be used in situations where depressing of the fast Na^+ channels is beneficial. Non-limiting examples of such situations include arrythmia, local anaesthetic, and pain. The compound can also br used in combination with drugs acting on fast sodium channel such as lidocaine.

EXAMPLE 13

Effect of MV8612 on L-type Ca^{2+} current

In another series of experiments (n=4), we tested the effect of MV8612 on the L-type Ca^{2+} current (I_{Ca}) in chick embryonic heart cells using the whole-cell voltage clamp technique referred to above. Superfusion with 10^{-9}M of MV8612 decreased I_{Ca} amplitude by 10% and increasing the concentration of the compound up to 10^{-8}M further decreased the current amplitude by 25%. Higher concentration of MV8612 (10^{-7}M) decreased the I_{Ca} amplitude by 62%. These results showed that MV8612 possesses a more potent L-type Ca^{2+} channel antagonist properties than that of MV8608.

The relatively high depressing effect of the L-type Ca^{2+} channel by MV8612 would be beneficial where L-type Ca^{2+} blockade are usually used such as, for example, ventricular tachycardia and hypertension.

EXAMPLE 14

Effect of MV8612 on R-type Ca^{2+} channel

In another series of experiments (n=7), the effect of 10^{-7}M of MV8612 was tested on the R-type Ca^{2+} in human aortic VSM cells, using the single channel cell attached recording technique and intra and extra patch pipette application of drugs. As for MV8608, the R-type Ca^{2+} channel was recorded in the presence of 10^{-6}M of the L-type Ca^{2+} channel blocker nifedipine (in the patch pipette) and using extra-patch pipette solution (containing 140mM KCl) that mimics the intracellular ionic concentration (to zero the extra-pipette cell membrane potential).

In one series of experiments (n=3), MV8612 (total concentration, 10^{-7}M) was only applied to the patch pipette. As shown in Figures 27A-C, application of 10^{-7}M MV8612, via the patch pipette, significantly decreased the R-type single Ca^{2+} channel current amplitude (by about 75% of the control value panel A) and the probability and the opening time of the R-type Ca^{2+} channel (Panel B and C). As for MV8608, in one series of experiments (n=4), MV8612 (10^{-7}M) was only applied to the extra-patch pipette, in order to verify if the compound penetrates into the cytosol and if so, how it affects the R-type Ca^{2+} channel activity. As shown in Figures 27D and E, extra-patch pipette application of MV8612 rapidly increased the R-type Ca^{2+} opening frequence and was accompanied by a small decrease in the single channel current at all sustained membrane potential (HP) levels used (Figure 27 shows example at HP of -30 where control channel opening is high and a +10mV where control channel opening is low).

These results demonstrate that as for MV8608, intra-patch pipette application of MV8612, equivalent to extracellular application in normal

working single cells or muscle, decreased both R-type Ca^{2+} channel amplitude as well as the probability and duration of opening thereof. However, the extend of blocking of the R-type Ca^{2+} channel amplitude and probability of opening by MV8612 was superior to that of MV8608. Also, these results demonstrate that as MV8608, MV8612 did penetrate the cytosol and did increase the frequency of opening (but not the opening-time) of the channel. However, unlike MV8608, intracellular MV8612 permanently depressed the amplitude of the R-type Ca^{2+} channel. The increase of frequency of opening of the R-type channel by intracellular MV8612 would allow extracellular MV8612 to block the channel activity at the opening state. The large decrease of the R-type Ca^{2+} channel amplitude and activities by intra-patch pipette application of MV8612 when compared to the effect with MV8608 could be due to the permanent decrease of the R-type Ca^{2+} channel by intracellular MV8612 but not by MV8608.

In summary, MV8612 seems to be a more effective blocker of the R-type Ca^{2+} channel than that of MV8608 and this difference is mainly due to the permanent depressing effect of the R-type Ca^{2+} channel by intracellular MV8612. Experiments using $[Ca]_i$, $[Ca]_c$ and $[Ca]_n$ as well as *in vivo* work (see below) confirm these results and show a more potent effect of MV8612 on the R-type Ca^{2+} channel when compared to that of MV8608.

Thus, the high potency blockade of the R-type Ca^{2+} channel by MV8612 will be more effective than MV8608 in reducing Ca^{2+} overload that occurred during many abnormal cell function such as those described for MV8608 in example 10. Also, since MV8612 (but not MV8608) depressed the fast Na^+ channel and the L-type Ca^{2+} channel, this compound will be highly effective not only in acute but also in chronic diseases such as those described in examples 10 to 13.

EXAMPLE 15

Effect of MV8612 on $[Ca]_i$, $[Ca]_c$, $[Ca]_n$ in the presence of extracellular L-type Ca^{2+} blocker

In another series of experiments, as for MV8608, the effect of MV8612 was tested on $[Ca]_i$, $[Ca]_c$, $[Ca]_n$ (in the presence of extracellular L-type Ca^{2+} blocker, nifedipine, 10^{-6} M) of embryonic chick heart (Figures 29 to 31 and 34) and human aortic VSM cells (Figures 29 to 33) as well as *in vivo* anaesthetized guinea pig (Figure 36).

Using Fura-2 $[Ca]_i$ measurement technique, in one series of experiments we tested the effect of MV8612 (10^{-9}M) on the stimulation of R-type Ca^{2+}-induced sustained increase of $[Ca]_i$ by sustained depolarization (Figures 26 and 27), PAF (10^{-9}M, Figure 34) and ET-1 (Figures 33 and 34). These experiments showed that 10^{-9}M of MV8612 significantly decreased sustained increase of $[Ca]_i$ induced by sustained depolarization and hormones. Also, MV8612 (10^{-8}M) was found to prevent stimulation of the R-type Ca^{2+} channel induced sustained increase of $[Ca]_i$ by sustained depolarization and chronic concentration of PAF (10^{-7}M) (Figure 32). Using 3-dimension Fluo-3 Ca $^{2+}$ measurement of $[Ca]_c$ and $[Ca]_n$, MV8612 was found to be a more potent blocker than MV8608 in the overstimulation of R-type Ca^{2+} channel induced sustained increase of $[Ca]_c$ and $[Ca]_n$ by sustained depolarization and high concentration of PAF (10^{-7}M) (Figures 29 and 30). MV8612 was also found to be equipotent in preventing the stimulation of the R-type Ca^{2+} channel by sustained depolarization and high concentration of PAF (10^{-7}M) (Figure 31).

On the other hand, *in vivo* administered PAF, induced a marked hypotension in the anaesthetized rat and guinea pig. In addition, the pro-inflammatory mediator also induced an important bronchoconstriction in the guinea pig, where PAF induced its hypotensive effects through the release of EDRF, its bronchoconstrictive properties are solely mediated by the release of thromboxane A_2.

Characteristically, standard Ca^{2+} blockers such as nifedipine and the dual L and R-type blocker isradipine have marked intrinsic hypotensive properties in the rat (results not shown) and in the guinea pig (Figure 35).

Interestingly, the R-type blocker, MV8612, is devoid of marked intrinsic effect in the guinea pig. As shown in Figure 36, pretreatment of the guinea pig with MV8612 abolished the bronchoconstrictive responses and very significantly reduced the hypotensive effects of PAF. Following withdrawal of the treatment with MV8612, the hypotensive effect of PAF is fully restored in the same animal (for methodology, please refer to Gratton et al., 1995a, Am. J. Hyper. 8:1121-1127). Identical inhibition of MV8612 has been observed on the hypotensive effect of PAF in the anaesthetized rat (for methodology, please refer to D'Orléans-Juste et al., 1996, Can. J. Physiol. Pharmacol. 74:811-817; Gratton et al., 1995a, *supra*; 1995b, Br. J. Pharmacol. 114:720-726).

These results again confirm the more potent effect of MV8612 (when compared to MV8608) on blocking the R-type Ca^{2+} channel and related cytosolic and nuclear Ca^{2+} overload. The high potency effect of MV8612 of the R-type Ca^{2+} combined to its depressing effect of the fast Na^+ and the L-type Ca^{2+} channels give this compound a higher spectrum of action than that of MV8608 and isradipine. The MV8612 as well as MV8608 are unique compounds that block both the cytosolic and nuclear Ca^{2+} overload. This later effect of MV8608 and especially MV8612 gives these compounds a new target other than that of the cytosolic membrane channels but also a nuclear and a perinuclear ionic channel target blockers. The MV8612 will be beneficial in cell disorders that implicate abnormal Ca^{2+} and Na^+ transport and preventing cytosolic Ca^{2+} overload such as in diseases cited in examples 7 to 14.

In conclusion, the pharmacological results presented herein support the unique R-type Ca^{2+} channel blocking properties of the MV8608 and MV8612 and their derivatives.

EXAMPLE 16

"In vivo" results with compounds MV8608 and MV8612

In this experiment (N =5 to 6 animals per group), the anti-oedemagenic action of compounds MV 8608 and MV 8612 against bradykinin and several mediators which are reported to be involved in the inflammatory processes, was evaluated. The procedures used to perform these experiments have been reported elsewhere (Neves et al., 1993, Eur. J. Pharmacol. 243:213-219; Campos et al., 1995, Br. J. Pharmacol. 114:1005-1013; Campos et al., 1996, Br. J. Pharmacol. 117:793-798; Eur. J. Pharmacol. 316:227-286). As can be seen in Figure 37A, MV 8608 (10 and 100 nmol/paw), when co-injected with des-Arg^9-bradykinin, caused a dose-related inhibition of paw oedema induced by this peptide. In contrast, at similar doses, MV 8608 had no significant effect against bradykinin-induced hindpaw oedema in animals treated with LPS (Campos et al., 1996, *supra*) (Figure 37B). As reported previously, in rats treated 30 days prior to a systemic injection of LPS, both B_1 and B_2 kinin selective agonists caused marked oedema formation (Figures 37C and D (Campos et al., 1996, *supra*)). Under such experimental conditions, compound MV 8608 (100 nmol/paw) significantly inhibited both des-Arg^9-BK and bradykinin-induced rat paw oedema (Figures 37C and D). However, MV8608 was more effective against B_1 agonist-mediated oedema formation. In addition, at 100 nmol/paw, compound MV 8608 consistently inhibited the paw oedema induced by PAF (Figure 38B), and partially inhibited the oedema induced by prostaglandin E_2 (PGE_2) (Figure 38A), leaving oedema induced by substance P unaffected (Figure 38C). Interestingly, MV 8608 (10 and 100 nmol/paw) also inhibited significantly oedema formation induced by subplantar injection of ovalbumin in animals that had been actively sensitised to this antigen (Figure 38D).

Results of Figure 39 (A and B) demonstrate that MV 8608 (10 and 100 nmol/paw) in a dose-dependant fashion prevented the potentiation of paw oedema caused by association of low dose of des-Arg^9-BK plus PAF

(Figure 39A) or with PGE_2 (Figure 39B). MV 8608 (10 and 100 nmol/paw) when co-injected in association with carrageenan (Figure 40A), dextran (Figure 40B), histamine (Figure 40C) or with serotonin (Figure 40D), significantly prevented the oedemagenic response caused by these substances. However, MV 8608 was much more effective in inhibiting paw oedema induced by carrageenan, an effect which has been reported to be dependent on the release of several mediators, including kinins, histamine, serotonin and PAF (Hargreaves et al., 1988, Clin. Pharmacol. Ther., 44:613-621; Burch et al., 1990, Naunyn-Schimiedeberg's Arch. Pharmacol., 342:189 -193; Damas et al., 1992, Eur. J. Pharmacol., 211:81-86; Damas et al., 1996, Naunyn-Schmiedeberg's Arch. Pharmacol., 354:670-676; De Campos et al., 1996, Eur. J. Pharmacol. 316:227-286).

In marked contrast, and confirming previous "in vitro" and "in vivo" results (Calixto et al., 1986, Br. J. Pharmacol. 88:937-941; Calixto et al., 1985, Br. J. Pharmacol., 85:729- 731; 1987, *supra*; 1988, *supra*; 1991a, Prostaglandins, 41:515-526; 1991b, In: Bradykinin Antagonists: Basic and Clinical Research. Ed. by Ronald M. Burch, Marcel Dekker Inc, New York, pp.97-129), MV 8612 (10 and 100 nmol/paw) significantly inhibited bradykinin and the selective B_2 agonist tyr^8-bradykinin-induced paw oedema (Figures 41A and D), while having no significant effect against oedema-induced by des-Arg^9-BK in animals treated with LPS (Figure 41B) (Campos et al., 1996, *supra*). The anti-oedematogenic effect caused by MV 8612 seems to be systemic, because the controlateral paw treated with saline also revealed an significant anti-oedemagenic action (Figure 41C). On the other hand, MV 8612 (10 and 100 nmol/paw) dose-dependently inhibited PGE_2 and carrageenan-induced oedema formation (Figure 42A, C), but caused only a minimal inhibition of PAF and substance P-mediated paw oedemas (Figures 42B and D). Compound MV 8612 (10 nmol/paw) significantly inhibited the oedema formation caused by association of low dose of bradykinin plus the calcitonin gene related peptide (Figure 43A), PGE_2 (Figure 43B) and prostacyclin (Figure

43D), but did not interfere with oedema-induced by the association of bradykinin plus PAF (Figure 43C). The anti-oedematogenic action of MV 8612 against bradykinin-induced oedema was independent on the release of histamine, since a dose-related inhibition was still observed in animals treated with cyprohetadine or with compound 48/80 (Figures 44A and B). The inhibition of MV 8612 against oedema caused by bradykinin installs rapidly (30 min) and lasted for at least 2 h (Figure 45).

When tested in mice, MV 8612 (40 to 160 nmol/paw) inhibited bradykinin and carrageenan-induced paw oedema in mice in a dose-dependent manner (Figures 46A and B). At the same dose, MV 8612 failed to affect significantly PAF-acether or serotonin-induced oedema formation (Figures 46C and D). Confirming the effect observed with crude extract of *Mandevilla velutina*, when MV 8612 was injected systemically, MV 8612 (7.5 and 15 mol/kg, i.p.), given 30 min prior, produced a dose-dependent inhibition of bradykinin and cellulose sulphate-induced paw oedema (Figure 47A and B). However, at the same range of dose, MV 8612 had no significant effect against paw oedema induced by serotonin and histamine (Figure 47C and D). In marked contrast, compound MV 8608 (7.5 and 15 mol/kg, i.p) caused a dose-related inhibition of histamine and serotonin-induced oedema formation, leaving paw oedema to bradykinin unaffected (Figure 48A ,B and C).

Taken together, these results indicate that both MV 8612 and MV 8608 compounds show potent topical and systemic anti-inflammatory properties through a distinct mechanism of action, albeit through an overstimulation of R-type Ca^{2+} channels. While MV 8612 was more active against inflammatory response caused by bradykinin via stimulation of B_2 receptors, MV 8608 was effective in preventing oedemas elicited by histamine, PAF-acether and by the B_1 selective agonist des-Arg^9-BK.

EXAMPLE 17

Anti-inflammatory action of MV8612 and MV8608

To assess further the anti-inflammatory action of MV 8612 and MV 8608, their effects against the inflammatory responses caused by several mediators of inflammation in a murine model of pleurisy were tested (Saleh et al., 1996, Br. J. Pharmacol. 118:811-819). In addition, both compounds were tested against the increase of vascular permeability caused by bradykinin in the rat skin (Neves et al., 1993b, Phytotherapy Research. 7:356-362). Figure 49 shows that compound MV 8612 (30 and 60 mol/kg, i.p.) given 1 h prior, significantly inhibited plasma extravasation (A) as well as the total (B) and neutrophils cells (C) in response to intrapleural injection of carrageenan. Compound MV 8608 (30 mol/kg, i.p) also inhibited significantly the total and neutrophil cells migration caused by intrapleural injection of carrageenan (Figure 50). Confirming the previous results, compound MV 8608 (30 mol/kg, i.p.) also antagonised significantly the plasma extravasation and the mononuclear cells influx caused by intrapleural injection of PAF (Figure 51). In contrast, at the same range of dose, MV 8612 did not affect significantly the inflammatory response caused by PAF acether in a murine model of pleurisy (Figure 51). However, both MV 8612 (8 and 16 mol/kg, i.p) and MV 8608 (27 and 54 mol/kg, i.p.) antagonised in a dose-related manner, the increase of vascular permeability caused by bradykinin in the rat skin (Figure 52A and B). Compound MV 8612 was more active than MV 8608.

This data extend our previous results (Calixto et al., 1991a, *supra*; Zanini et al., 1992, Phytotherapy Research, 6:1-5; Neves et al., 1993b, *supra*) supportting the view that both MV 8612 and MV 8608 exhibit powerful anti-inflammatory properties.

EXAMPLE 18

Effect of MV8612 and MV8608 on human lymphocyte proliferation *in vivo*

In a separate series of experiments, compounds MV 8612 and MV 8608 were tested to analyze whether they interfered with human lymphocyte proliferation "in vitro". These experiments were carried out as reported previously (Moraes et al., 1996, Eur. J. Pharmacol. 312:333-339). The results of Figure 53(A and B) show that compound MV 8612 (0.02 to 0.32 M) and, to a lesser extent, MV 8608 (14.5 to 116 M) caused graded inhibition of human lymphocyte proliferation, with MV 8612 being about 570 fold more potent. These results may be explained by their above-demonstrated anti-inflammatory properties.

EXAMPLE 19

Antinociceptive actions of MV8612 and MV8608

The antinociceptive actions of compound MV 8612 and MV 8608 were investigated in different models of nociception in mice as reported previously (Vaz et al., 1996, J. Pharmacol. Exp. Ther. 278:304-312). Compound MV 8612 (0.25 to 2.5 mol/kg, i.p.), given 30 min prior, caused dose-dependent inhibition of acetic acid (Figure 54A) acetylcholine (Figure 55A) and kaolin (Figure 56A)-induced writhing response in mice. However, MV 8612 was about 2-fold more potent against kaolin-induced pain. In contrast, compound MV 8608 (2.7 to 27 mol/kg, i.p.) caused only partial or even no antinociceptive action when tested in the same model of pain. When compared with morphine and indomethacin (Table 6), MV 8612 was about 2-fold more potent when assessed in the kaolin-induced writhes. Given intracerebroventricularly (i.c.v) MV 8612 (4.2 to 42 nmol/site), like morphine (0.26 to 13 nmol/site), caused a dose-related antinociception when assessed against acetic acid-induced writhes (Figure 57A and B). MV 8612 was about 12-fold less potent than morphine.

These results indicate that MV 8612, exhibits potent antinociceptive actions, comparable to those of morphine and indomethacin. Its

antinociceptive property is elicited by its R-type Ca^{2+} channel blocking properties and is associated with its anti-bradykinin action, but appears to be unrelated to activation of opioid, β-adrenergic, serotonin or to the interaction with the nitric oxide pathway (results not shown).

Table 6 - Antinociceptive potencies of MV 8612, morphine and indomethacin in mice.

	ID_{50} µmol/kg, i.p			
DRUG	KAOLIN	ACETYLCHOLINE	ACETIC ACID	TAIL-FLICK
MV 8612	0.4 (0.3-0.6)	2.2 (2.0-2.3)	2.4 (1.9-2.6)	no effect
MORPHINE	1.4 (1.2-1.7)	1.4 (1.0-1.8)	1.3 (1.0-1.6)	6.0(5.7-6.4)
INDOMETHACIN	1.6 (1.4--1.8)	0.8 (0.5-1.0)	1.2 (0.9-1.4)	no effect

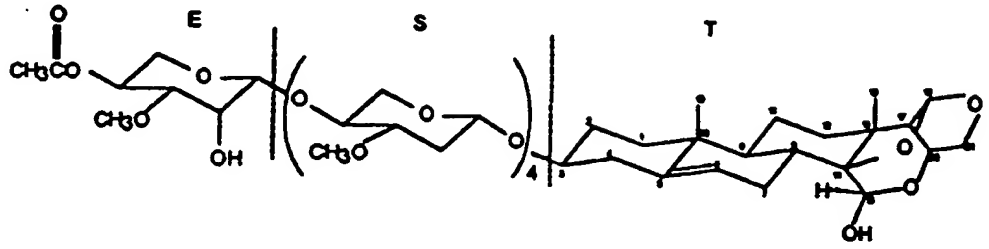
each group represents the mean of 6 to 8 animals

Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

The present description refers to a number of documents, the content of which is herein incorporated by reference.

WE CLAIM:

1. A compound having the general formula of MV8612 analogs VIIA and VIIB:



saponin-like derivatives thereof and pharmaceutically acceptable salts thereof.

2. A saponin-like compound having the general formula EST or a derivative of said sanopin-like compound, wherein E and S define a saponin oligosugar portion and T defines a steroid-like portion; wherein T is a pregnane-3β-ol derivative.

3. The compound of claim 2, wherein S is selected from the group comprising a tetra sugar derivative, a monomeric sugar derivative and an aligomeric of sugar derivatives.

4. The compound of claim 2 or 3, wherein S is selected from the group consisting of α(1-4) (2-deoxy, 3-methoxy) -L-lyxotetrose, α(1-4) (2-deoxy, 3-methoxy) L-xylotetrose, α(1-4) (2-deoxy, 3-methoxy)-L-arabinotetrose, α(1-4) (2-deoxy, 3-methoxy)-L-xylotetrose, α(1-4) (2-deoxy, 3-methoxy-L-ribopyranotetrose, α(1-4) (2-deoxy, 3 methoxy-L-sorbotetrose, α(1-4)-L-lyxotetrose, α(1-4)-L-xylotetrose, α(1-4)-L-arabinotetrose, α(1-4)-L-xylotetrose, α(1-4)-3, 4

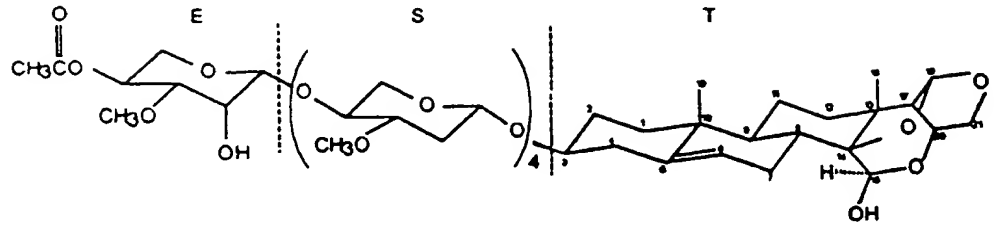
methoxy-L-sorbotetrose, α(1-4)-L-lyxotetrose, α(1-4)-L-xylotetrose, α(1-4)-L-arabinotetrose, α(1-4)-L-xylotetrose, α(1-4)-3, 4 methoxy-L-lyxotetrose, α(1-4)-3, 4 methoxy-L-xylotetrose, α(1-4)-3,4 methoxy-L-arabinotetrose, α(1-4)-3,4 methoxy-L-xylotetrose, α(1-4)-3,4 methoxy-L-ribopyranotetrose, α(1-4)-3,4 methoxy-L-sorbopyranotetrose, α(1-4)-L-lyxotetrose, α(1-4)-L-xylotetrose, α(1-4)-L-arabinotetrose, α(1-4)-L-ribopyranotetrose, and α(1-4)-L-sorbotetrose.

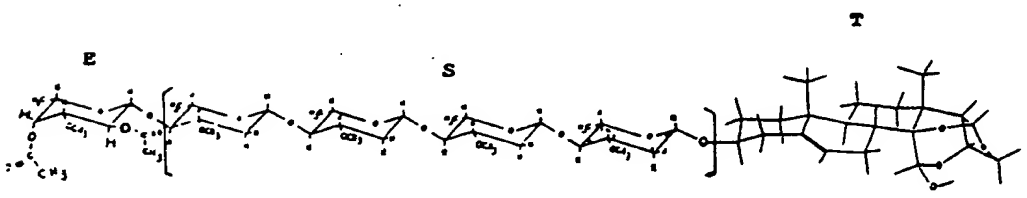
5. The saponin-like compound of claim 2, 3 or 4, wherein E is selected from the group consisting of 4-acetoxy-3 methoxy-L-α-lyxose, 4-acetoxy-3-methoxy-L-α-xylose, 4-acetoxy-3-methoxy-L-α-arabinose, 4-acetoxy-3-methoxy-L-α-xylose, -acetoxy-3-methoxy-L-α-ribopyranose, and 4-acetoxy-3-methoxy-L-α-sorbose-acetoxy.

6. The saponin-like compound of claim 2, 3, 4 or 5, wherein T is selected from the group consisting of 5-pregnane-3-ol oxytricyclo-15-ol, illustrol, 5-pregnane-3-ol-20-one, cholesterol, cholic acid, ergosterol, stigmasterol, androstenon, digitoxygenin, β-sitosterol, uvaol, ursolic acid, sarsasapogenin, 18,β -glycyrrhetinic acid, betulin, betulinic acid, oleanoic acid, and padocarpic acid.

7. The saponin-like compound of claim 1, 2, 3, 4, 5 or 6 wherein said compound and derivatives thereof are capable of displaying an inhibitory activity of the steady state R-type calcium channel.

8. A R-type Ca^{2+} channel blocker having the general formula of compound VIIA and compound VIIB:





and derivatives thereof.

9. A specific R-type calcium channel inhibitor having the general formula I **(IA and IB), II, III, IV, V, VI, VIIA and VIIB** indicated in Fig. 1 and Fig. 2.

10. The compound of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9, derivatized by one of alkylation, benzoylation, or glycosidation of the hydroxyl groups, chain of sugar extension or contraction.

11. A pharmaceutical composition for treating or preventing overstimulation of R-type Ca^{2+} channels associated with a disease or condition in a warm blooded animal, comprising at least one compound of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, together with a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of claim 11, wherein said compound does not significantly affect the basal activity of said R-type Ca^{2+} channel.

13. The pharmaceutical composition of claim 11, wherein said compound is MV8612 and/or MV8608.

14. A pharmaceutical composition for blocking or relieving side effects of a drug which overstimulate R-type Ca^{2+} channels comprising at least one compound of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, together with a pharmaceutical carrier.

15. The pharmaceutical composition of claim 14, wherein said compound is MV8608 and/or MV8612.

16. A pharmaceutical composition for the prevention or treatment of a disease or condition in which a sustained elevation of $[Ca]_c$, $[Ca]_n$ or R-type Ca^{2+} blocking is encountered, comprising at least one compound of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, together with a pharmaceutical carrier.

17. The pharmaceutical composition of claim 16, wherein said compound is MV8608 and/or MV8612.

18. A method for specifically inhibiting overstimulation of a R-type Ca^{2+} channel in a warm blooded animal comprising an administration of an effective amount of the compound of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, together with a pharmaceutically acceptable carrier.

19. The method of claim 18, wherein said compound is MV8612 and/or MV8608.

20. A method of treating or preventing a disease or condition associated with an overstimulation of R-type Ca^{2+} channels without significantly affecting the basal activity thereof comprising an administration of an effective amount of the compound of claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, together with a pharmaceutically acceptable carrier.

21. The method of claim 20, wherein said compound is MV8612 and/or MV8608.

22. A method of treating or preventing a disease or condition associated with a sustained elevation of $[Ca]_c$, $[Ca]_n$, R-type Ca^{2+} blocking, and/or cytosolic and nuclear Ca^{2+} accumulation, comprising an administration of a therapeutically effective amount of a R-type Ca^{2+} channel blocker compound according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 together with a pharmaceutically acceptable carrier.

23. The method of claim 22, wherein said compound is MV8612 and/or MV8608.

24. A method for decreasing proliferation of cancer and tumor cells comprising an incubation thereof with an effective amount of a R-type Ca^{2+} channel blocker compound according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, together with a pharmaceutically acceptable carrier.

25. The method of claim 24, wherein said compound is MV8612 and/or MV8608.

26. The compound of claim 1, 2, 3, 4, 5, 6 or 7, wherein said compound is capable of blocking cytosolic and nuclear Ca^{2+} overload.

27. The compound of claim 26, wherein said compound is MV8612 and/or MV8608.

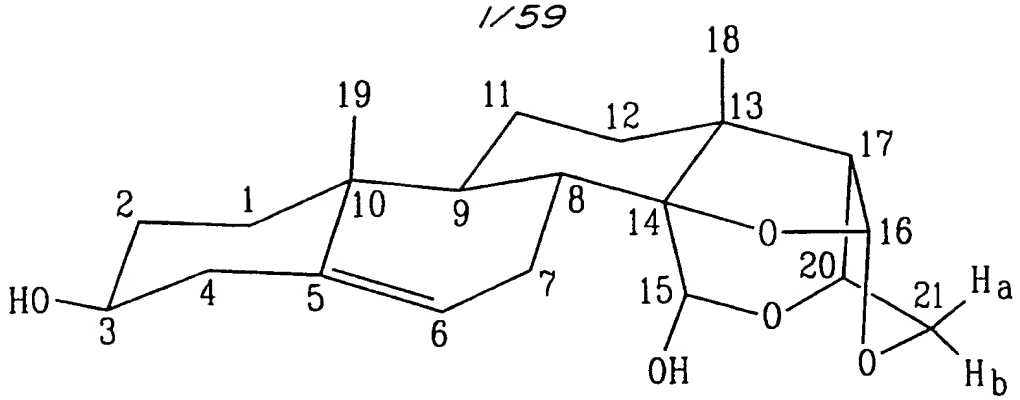


Fig - 1A

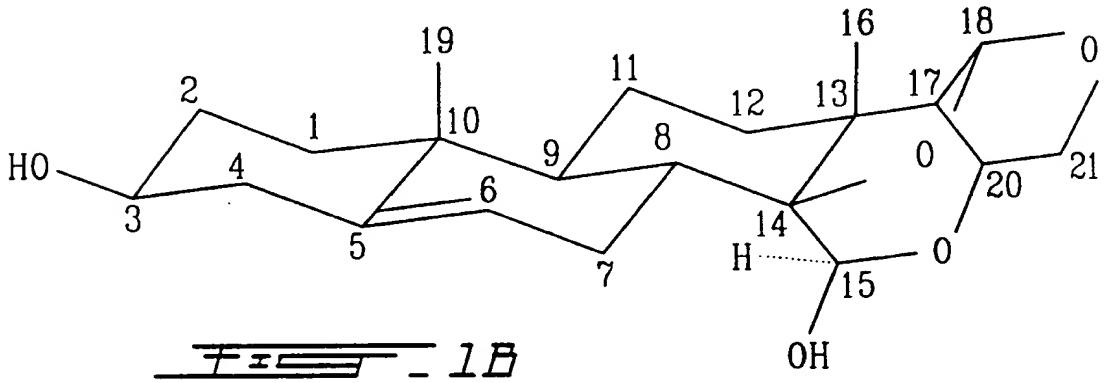


Fig - 1B

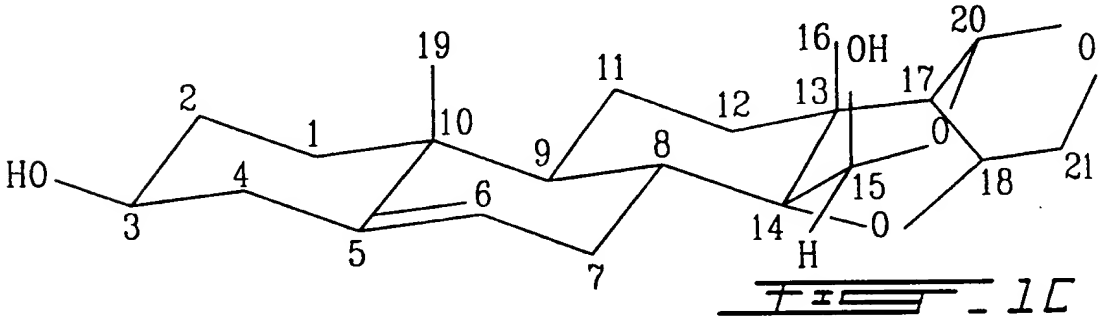


Fig - 1C

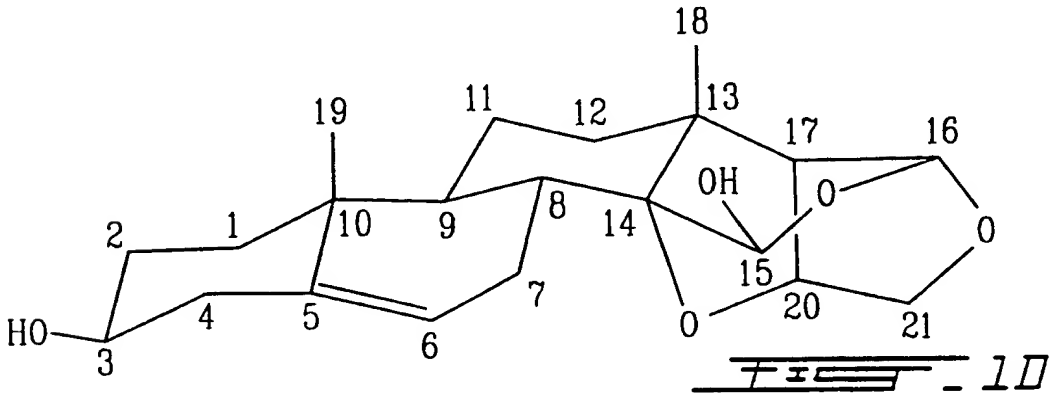


Fig - 1D

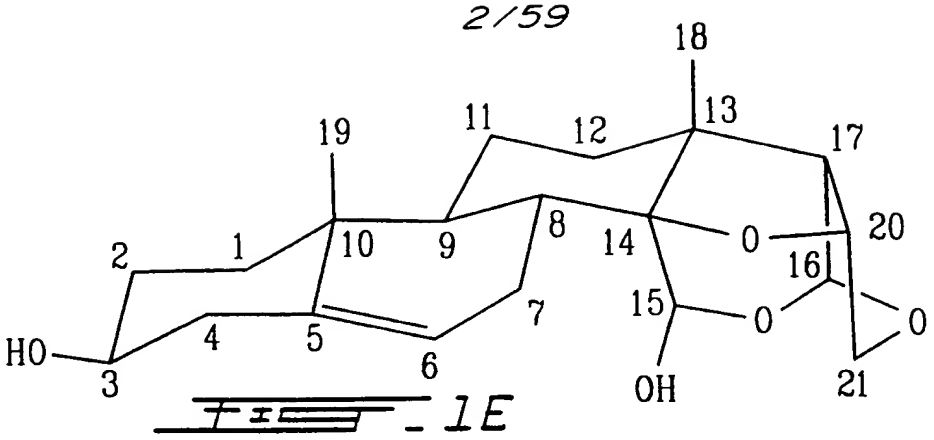


Fig - 1E

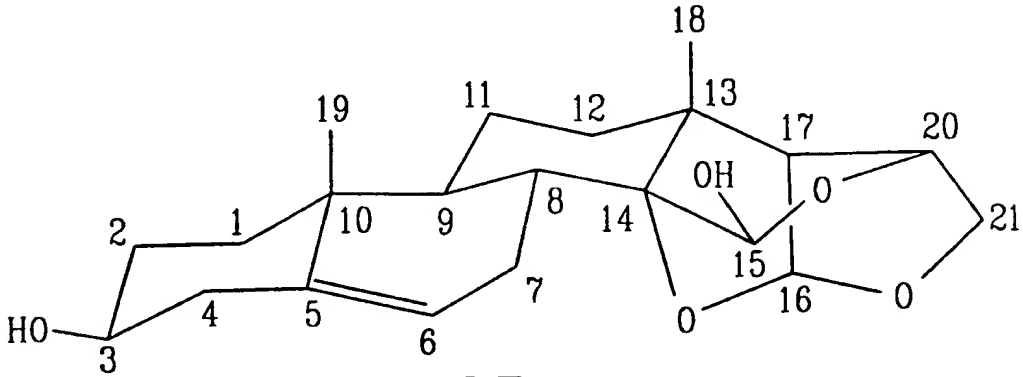


Fig - 1F

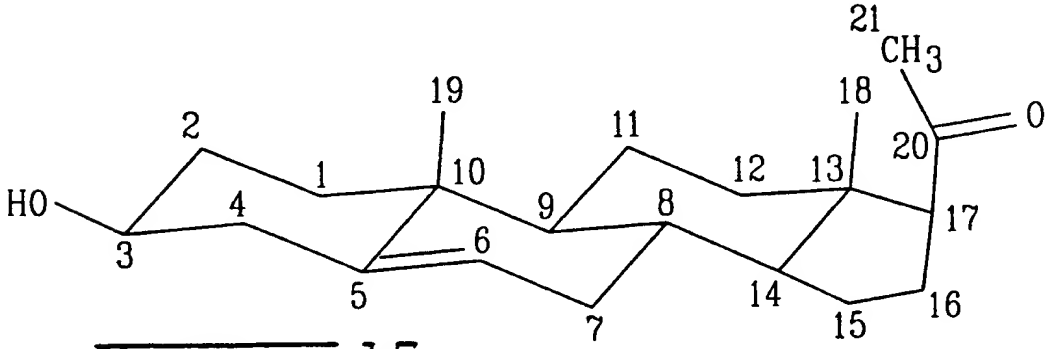


Fig - 1G

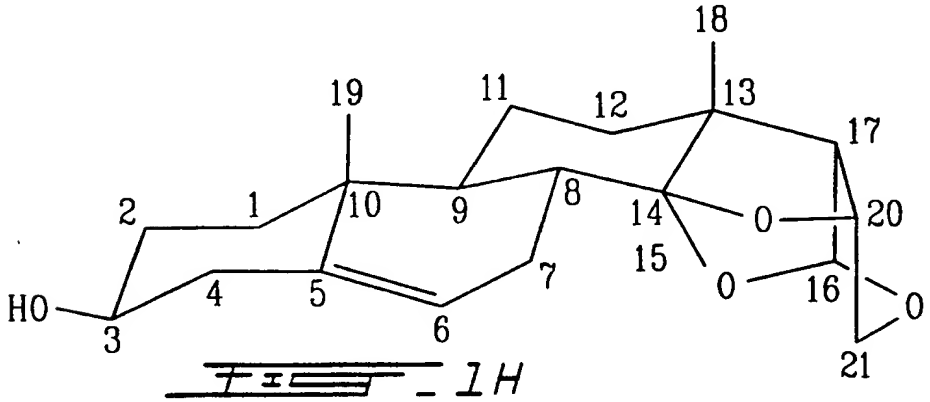


Fig - 1H

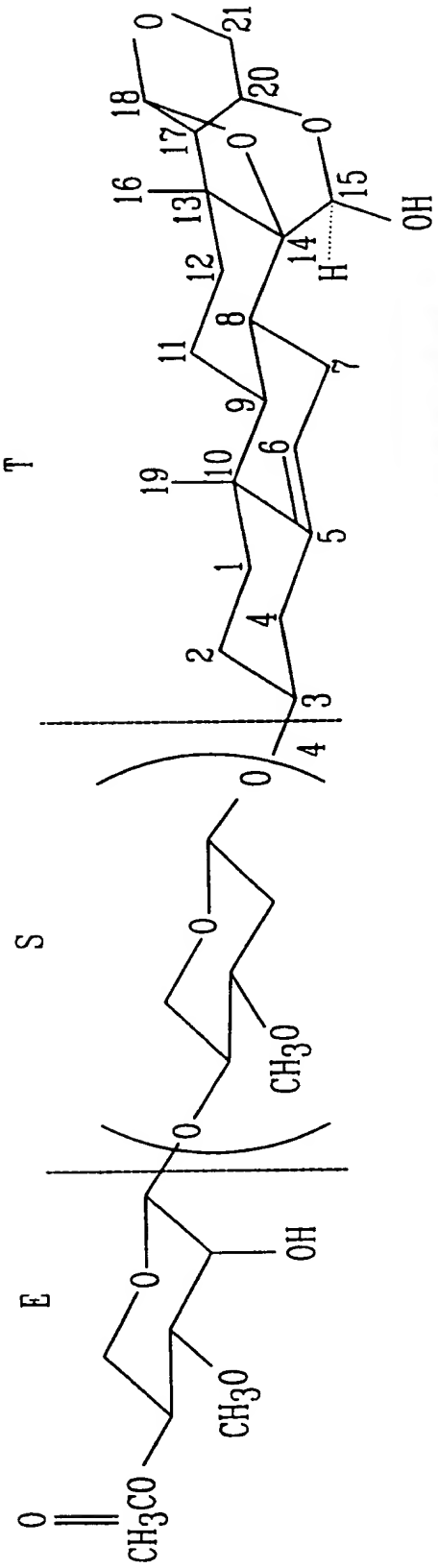


FIG - 2A

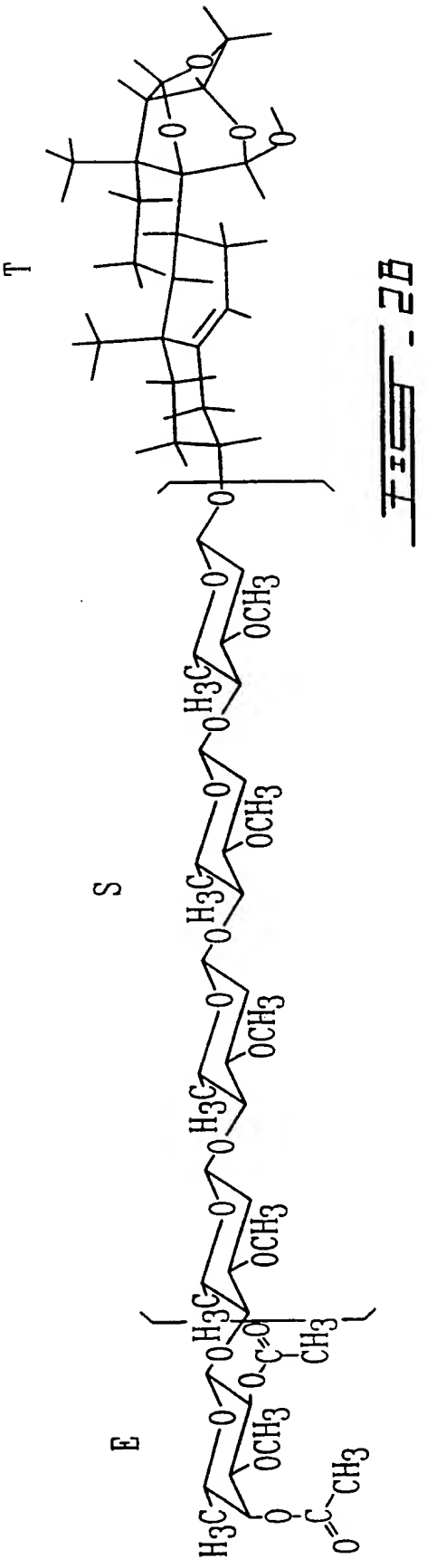


FIG - 2B

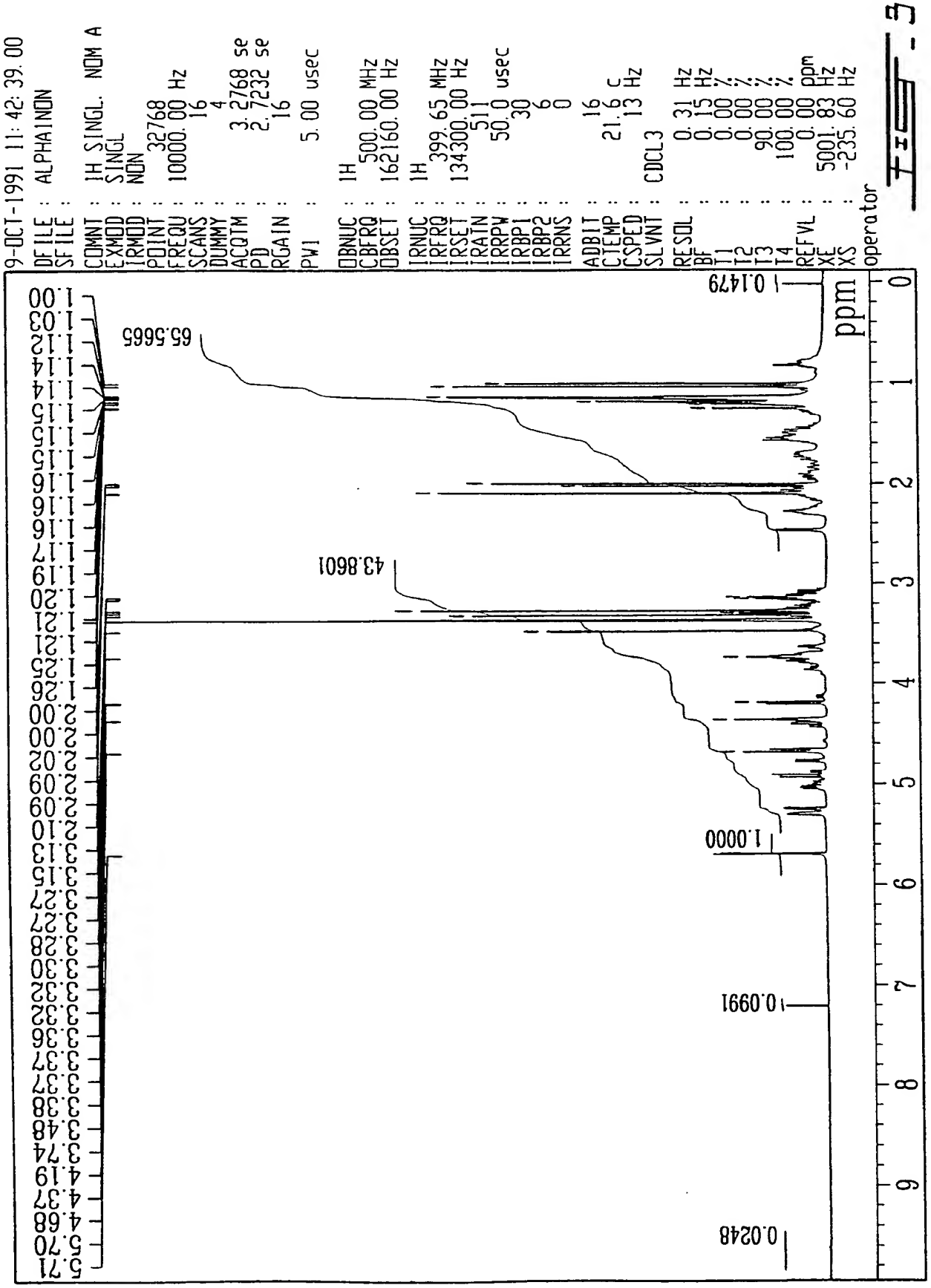

9-OCT-1991 11:42:39.00
DFILE : ALPHAINDN
SFILE :
COMNT : 1H SINGL. NDM A
EXMOD : SINGL
IRMOD : NDN
PDINT : 32768
FREQU : 10000.00 Hz
SCANS : 16
DUMMY : 4
ACQTM : 3.2768 se
PD : 2.7232 se
RGAIN : 16
PW1 : 5.00 usec
OBNUC : 1H
CBFRQ : 500.00 MHz
OBSET : 162160.00 Hz
IRNUC : 1H
IRFRQ : 399.65 MHz
IRSET : 134300.00 Hz
IRATN : 511
IRRPW : 50.0 usec
IRBP1 : 30
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 21.6 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 0.31 Hz
BF : 0.15 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 0.00 ppm
XE : 5001.83 Hz
XS : -235.60 Hz
operator
0.1479
65.5665
43.8601
1.0000
0.0991
0.0248
ppm
0
1
2
3
4
5
6
7
8
9

Fig - 3

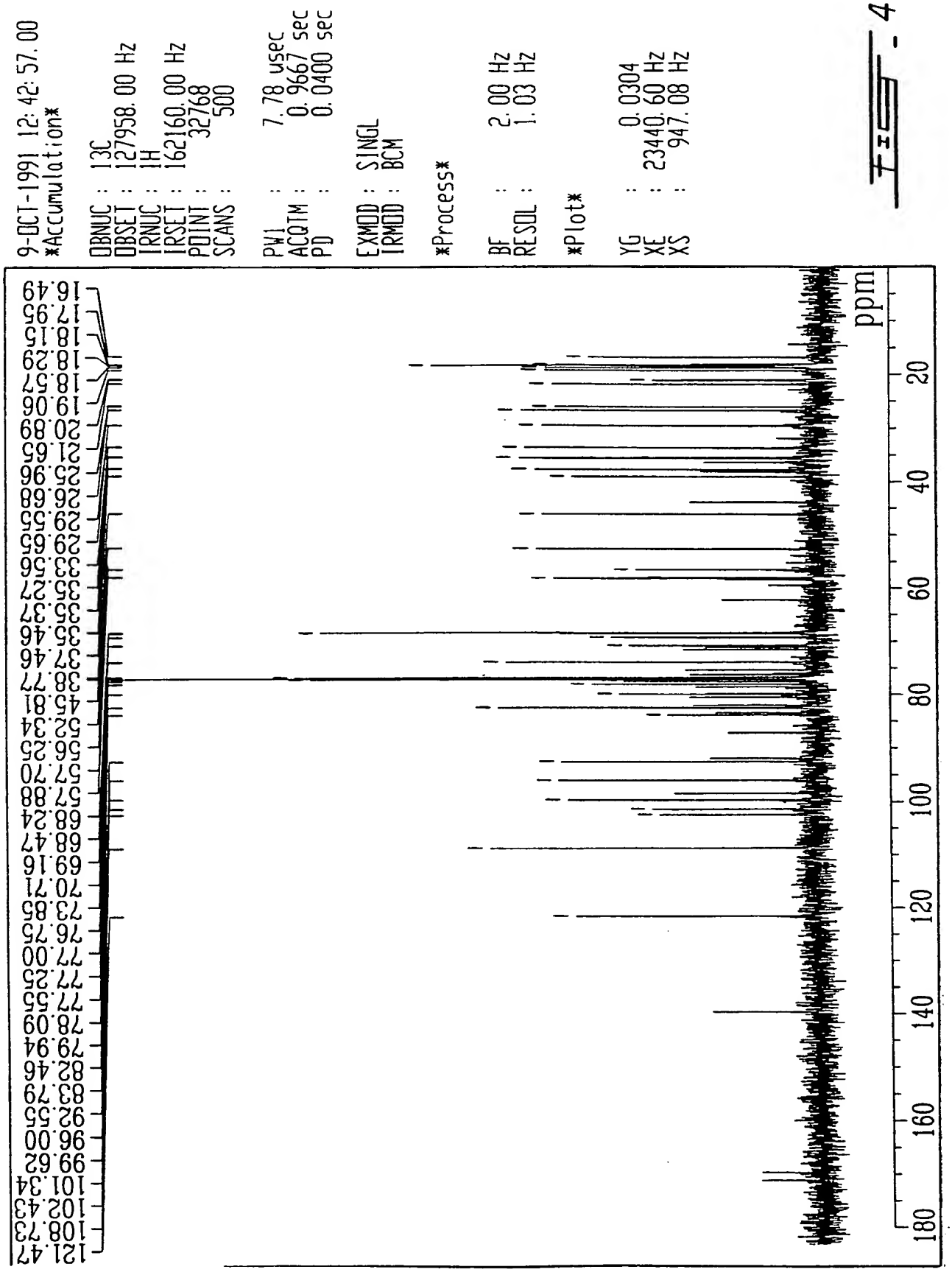

9-OCT-1991 12:42:57.00
Accumulation
OBNUC : 13C
OBSET : 127958.00 Hz
IRNUC : 1H
IRSET : 162160.00 Hz
POINT : 32768
SCANS : 500
PW1 : 7.78 usec
ACQTM : 0.9667 sec
PD : 0.0400 sec
EXMOD : SINGL
IRMOD : BCM
Process
BF : 2.00 Hz
RESOL : 1.03 Hz
Plot
YG : 0.0304
XE : 23440.60 Hz
XS : 947.08 Hz
ppm
20
40
60
80
100
120
140
160
180

FIG - 4

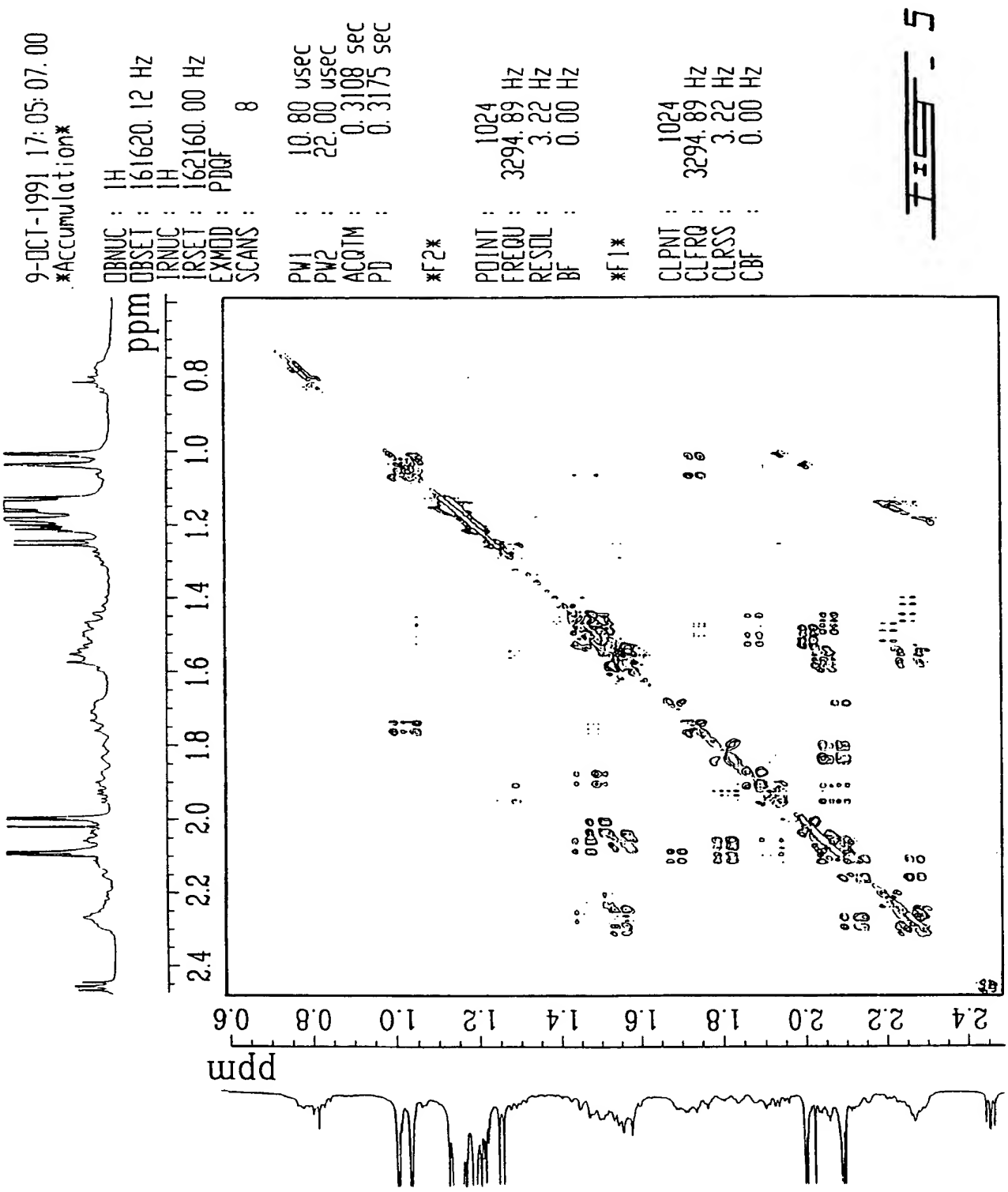

9-OCT-1991 17:05:07.00
Accumulation
OBNUC : 1H
OBSET : 161620.12 Hz
IRNUC : 1H
IRSET : 162160.00 Hz
EXMOD : PDQF
SCANS : 8
PW1 : 10.80 usec
PW2 : 22.00 usec
ACQTM : 0.3108 sec
PD : 0.3175 sec
F2
POINT : 1024
FREQU : 3294.89 Hz
RESOL : 3.22 Hz
BF : 0.00 Hz
F1
CLPNT : 1024
CLFRQ : 3294.89 Hz
CLRSS : 3.22 Hz
CBF : 0.00 Hz
ppm
2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8
ppm
2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8

FIG - 5

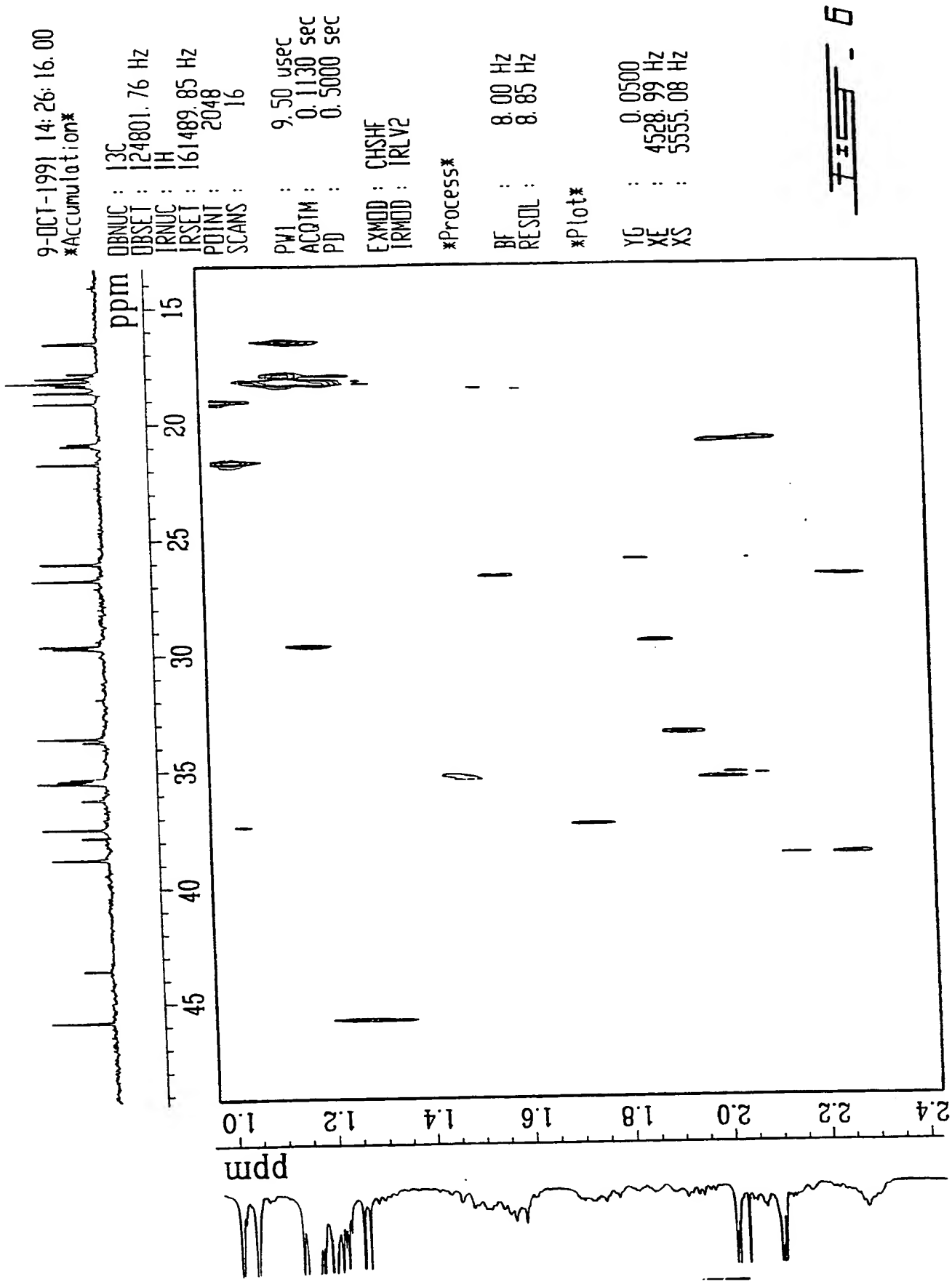
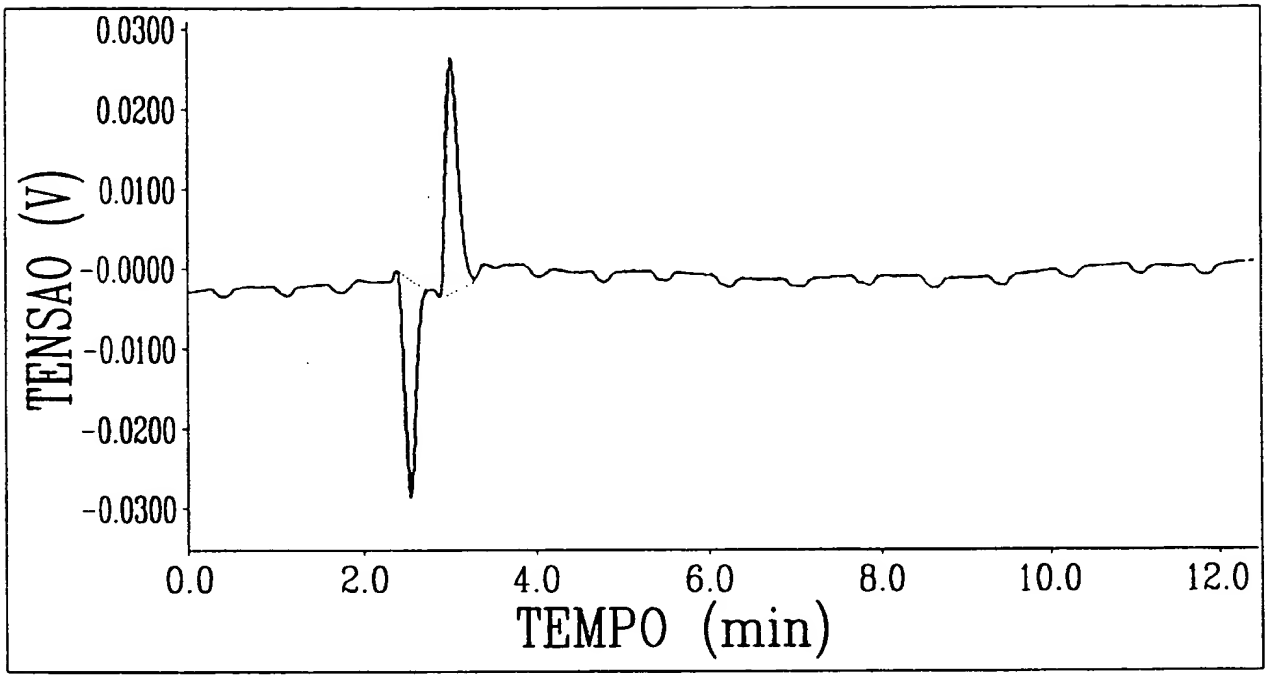
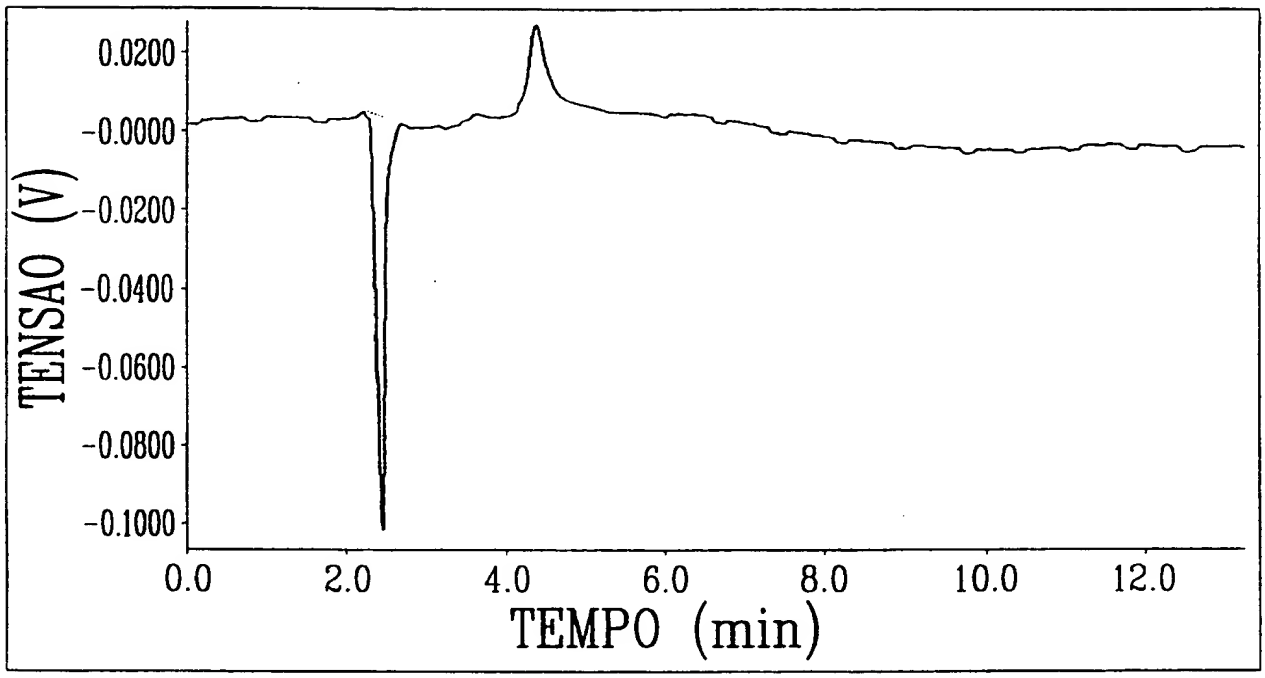

9-OCT-1991 14:26:16.00
Accumulation
OBNUC : 13C
OBSET : 124801.76 Hz
IRNUC : 1H
IRSET : 161489.85 Hz
POINT : 2048
SCANS : 16
PW1 : 9.50 usec
ACQTM : 0.1130 sec
PD : 0.5000 sec
EXMOD : CHSHF
IRMOD : IRLV2
Process
BF : 8.00 Hz
RESOL : 8.85 Hz
Plot
YG : 0.0500
XE : 4528.99 Hz
XS : 5555.08 Hz
ppm
15
20
25
30
35
40
45
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
ppm

FIG - 6



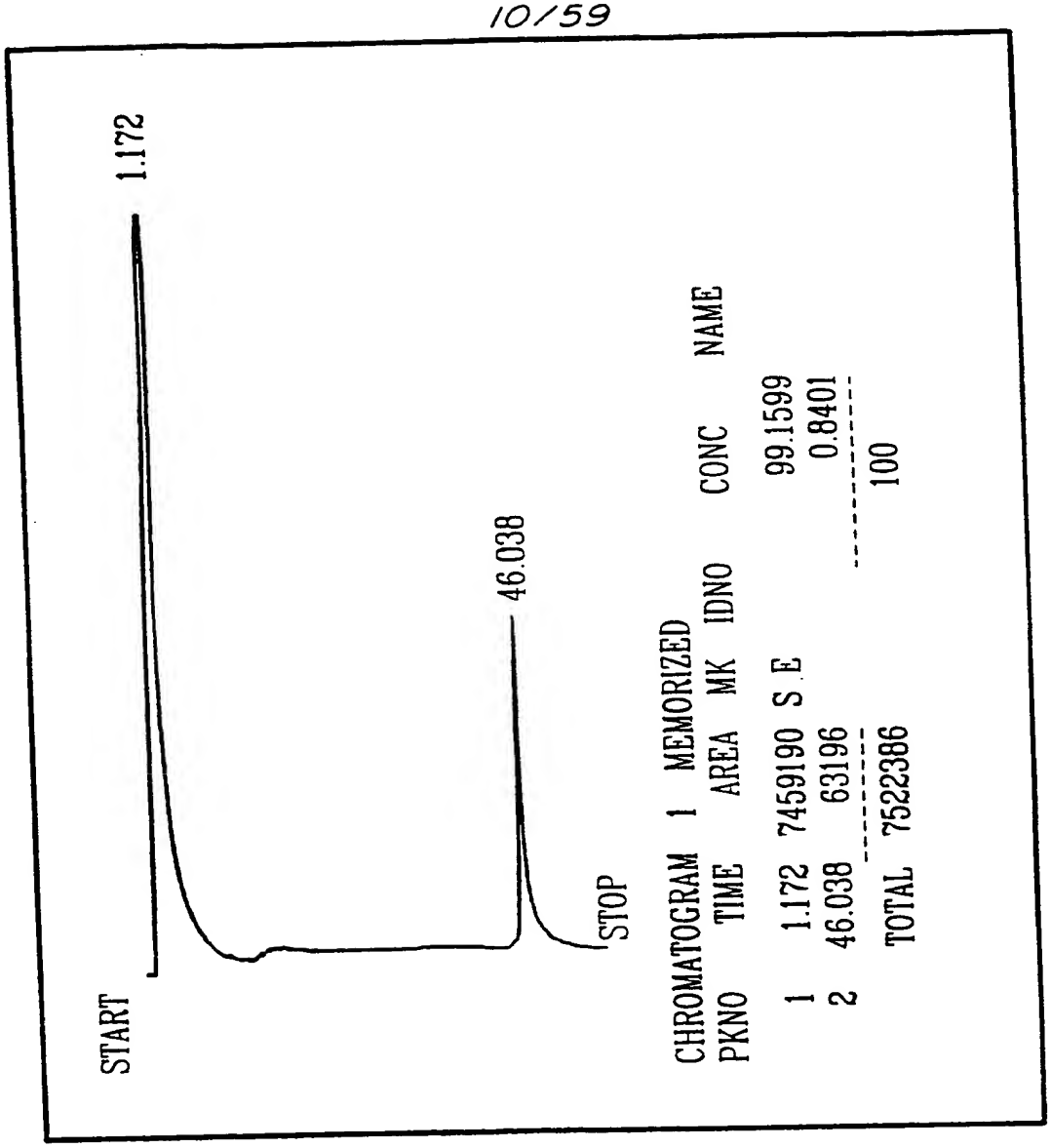
Peak 1. Solvent
Peak 2. MV-8608
Column Temperature 25°C
Chromatogram in HPLC (Beckmann)
Refractive Index Detector
Stationary phase → água/metanol

Fig. 7



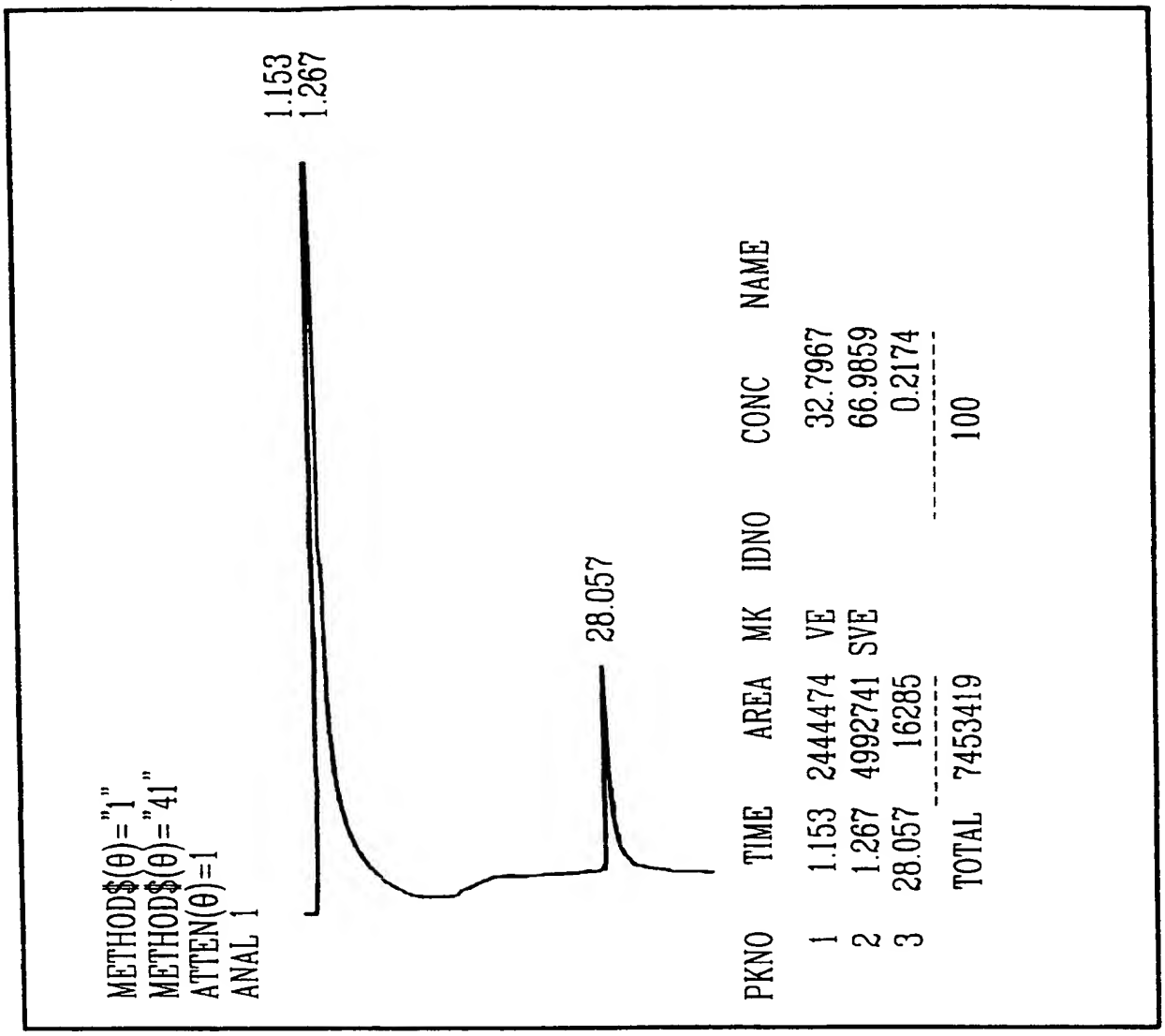
Peak 1. Solvent
Peak 2. MV-8612
Column Temperature 25°C
Chromatogram in HPLC (Beckmann)
Refractive Index Detector
Stationary phase → água/metanol

Fig 8



Chromatography → Shimadzu CG - 14A
Sample → MV 8608
Column temperature → 80°C → 250°C
Detector temperature → 290°C
Injector temperature → 250°C
Gradient temperature → 10°C/min
Column LM-1
Solvent → acetone
Peak 1 → solvent
Peak 2 → MV 8608

FIG - 9



Chromatography → Shimadzu CG - 14A
Sample → illustrol
Column temperature → 80°C → 250°C
Detector temperature → 290°C
Injector temperature → 250°C
Gradient temperature → 10°C/min
Column LM-1
Solvent → acetone/$CHCl_3$
Peak 1 → solvent
Peak 2 → solvent
Peak 3 → illustrol

FIG - 10

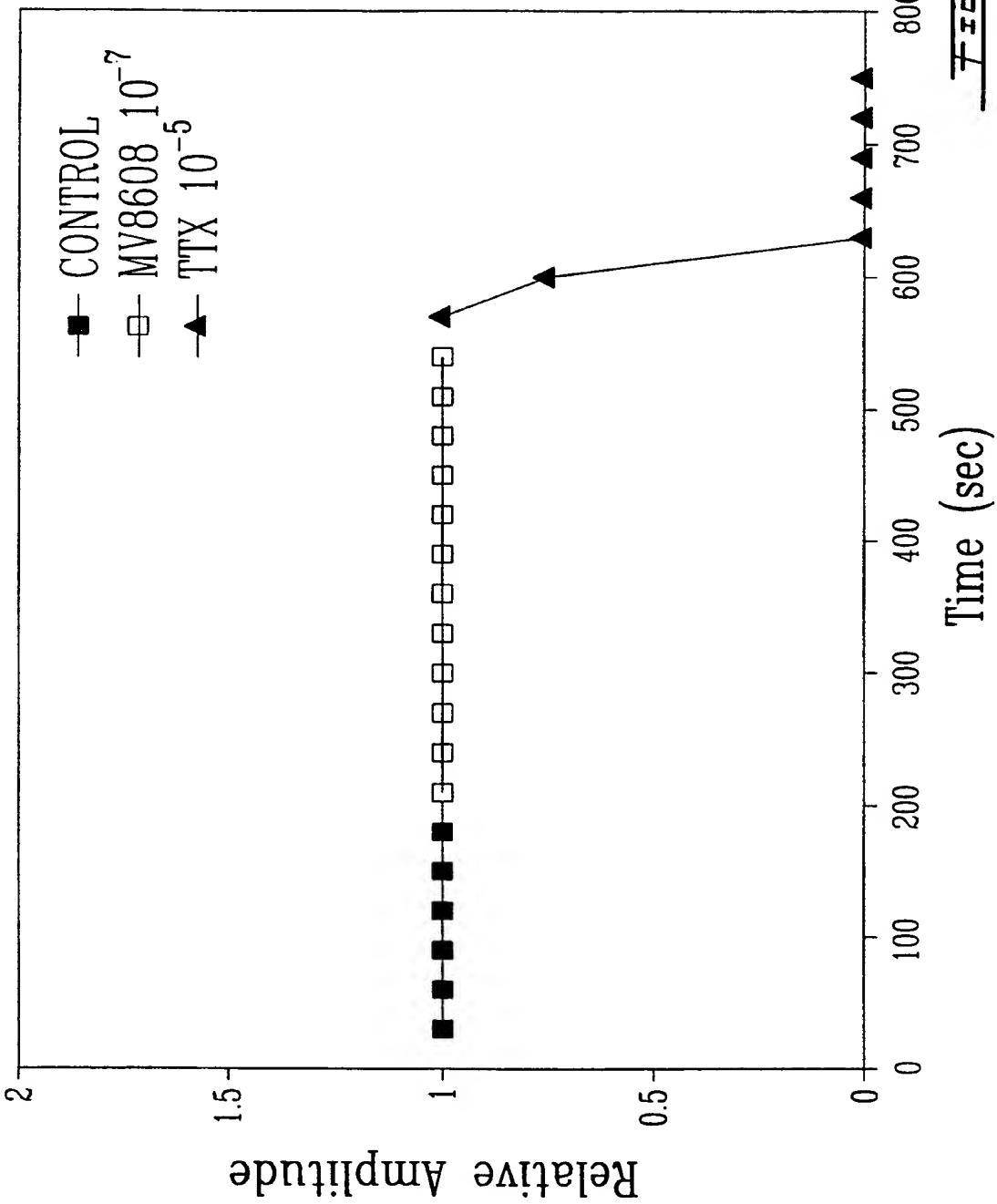

CONTROL
MV8608 10^{-7}
TTX 10^{-5}
Relative Amplitude
Time (sec)
0
0.5
1
1.5
2
0
100
200
300
400
500
600
700
800

Fig - 11

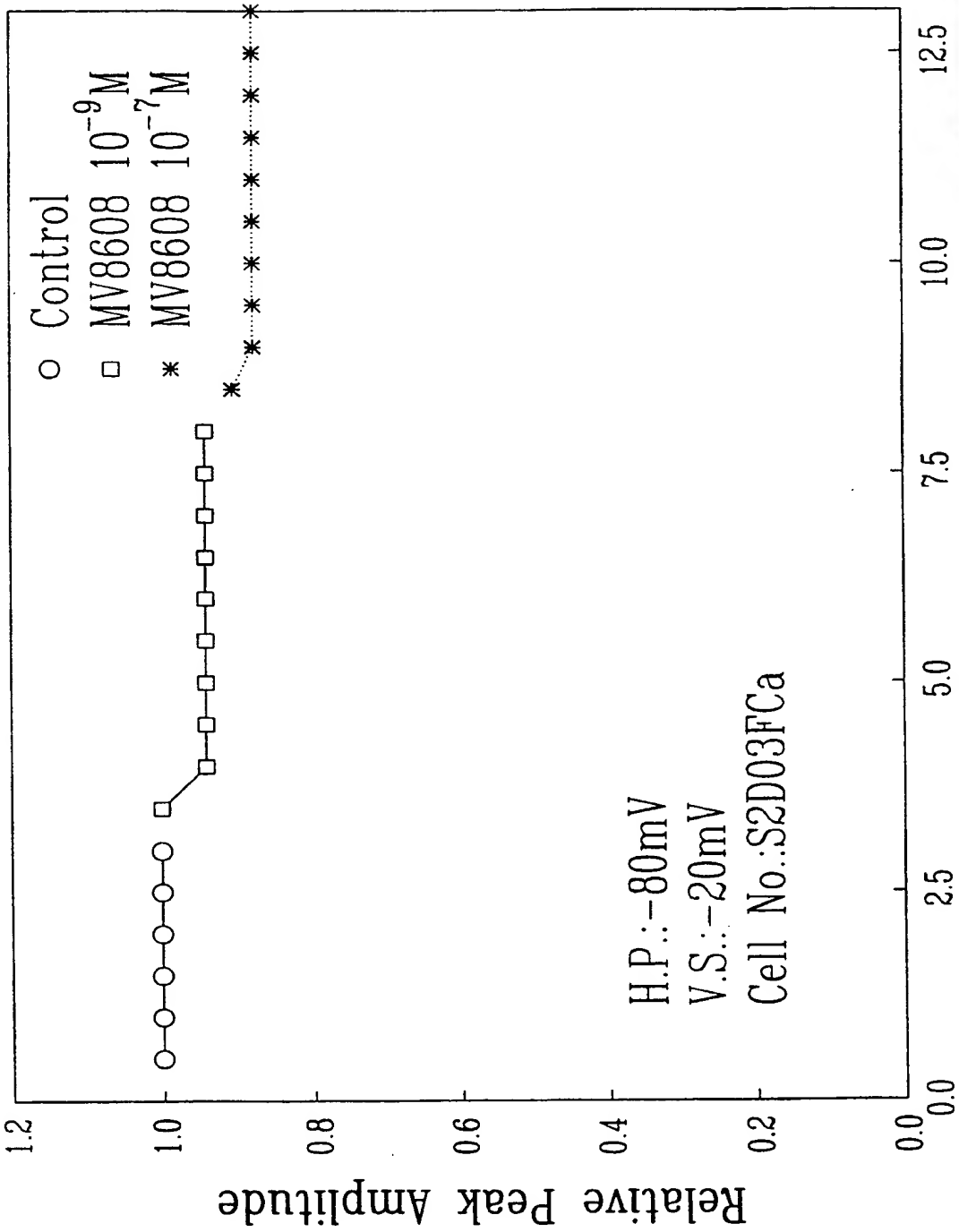

Relative Peak Amplitude
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
2.5
5.0
7.5
10.0
12.5
Time (min.)
Control
MV8608 10^{-9}M
MV8608 10^{-7}M
H.P.:-80mV
V.S.:-20mV
Cell No.:S2D03FCa

Fig-12

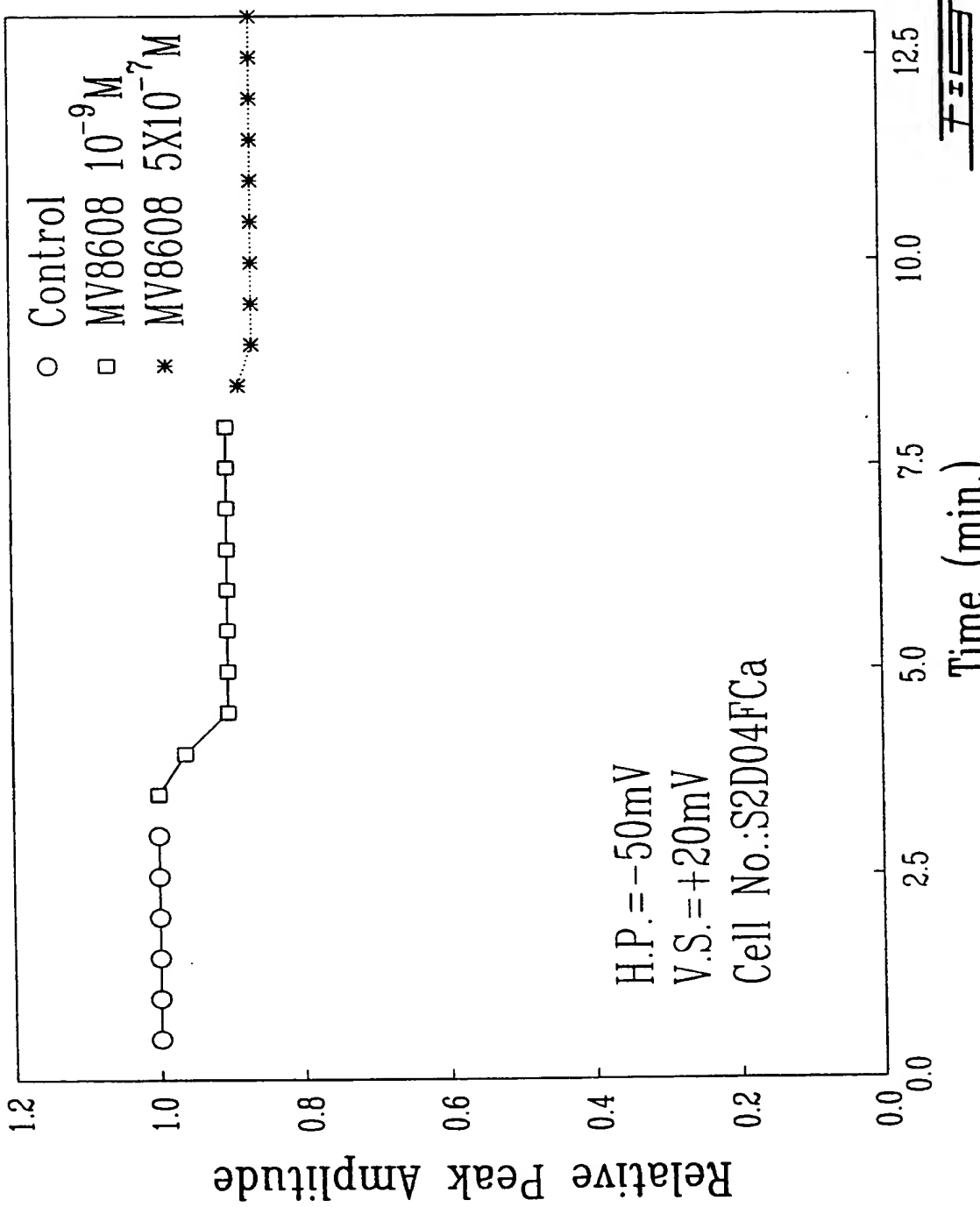

Relative Peak Amplitude
Time (min.)
Control
MV8608 10^{-9}M
MV8608 $5X10^{-7}$M
H.P.=-50mV
V.S.=+20mV
Cell No.:S2D04FCa
0.0
0.2
0.4
0.6
0.8
1.0
1.2
2.5
5.0
7.5
10.0
12.5

Fig. 13

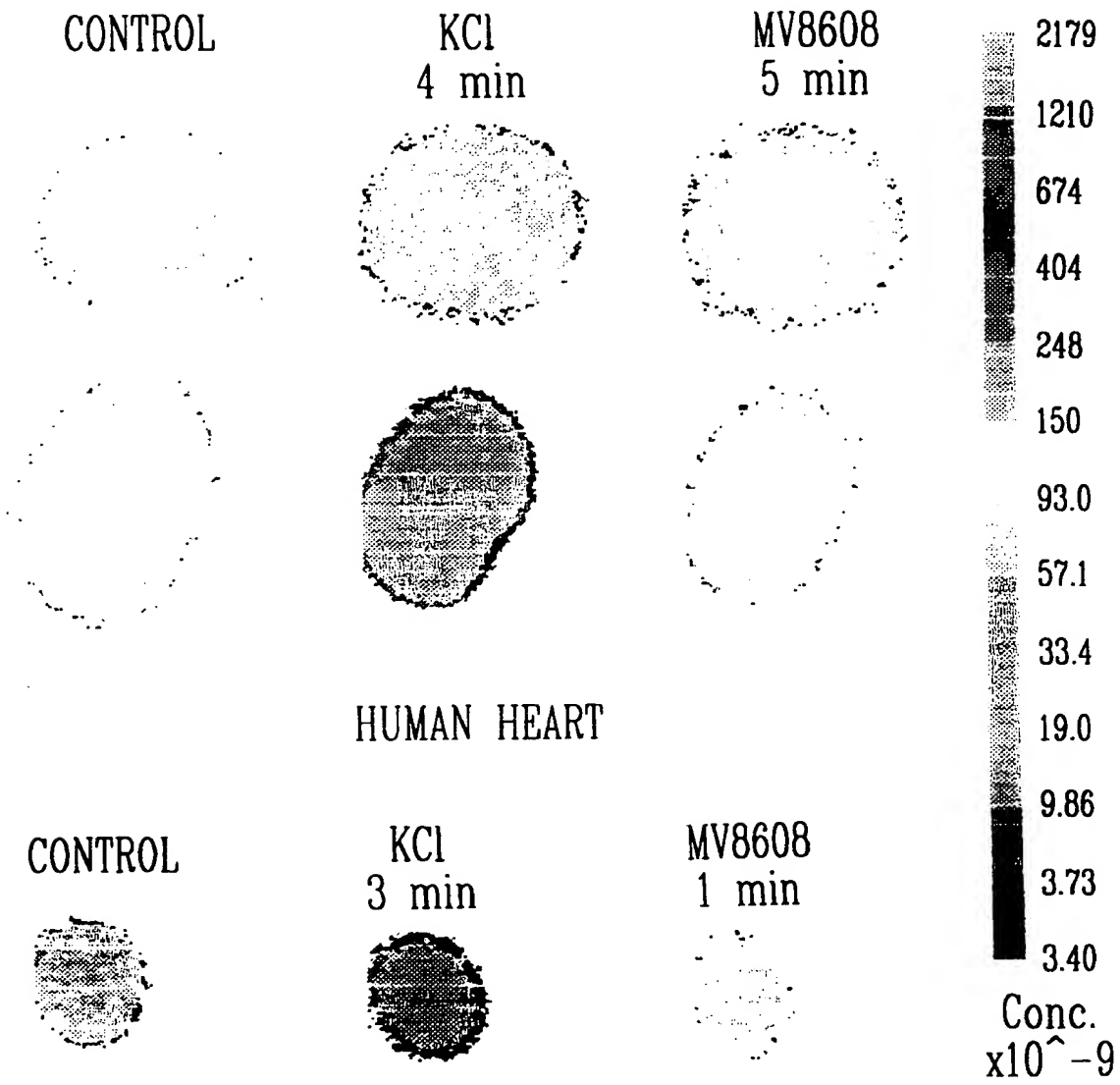

CONTROL
KCl
4 min
MV8608
5 min
HUMAN HEART
CONTROL
KCl
3 min
MV8608
1 min
2179
1210
674
404
248
150
93.0
57.1
33.4
19.0
9.86
3.73
3.40
Conc.
x10^-9

FIG. 15

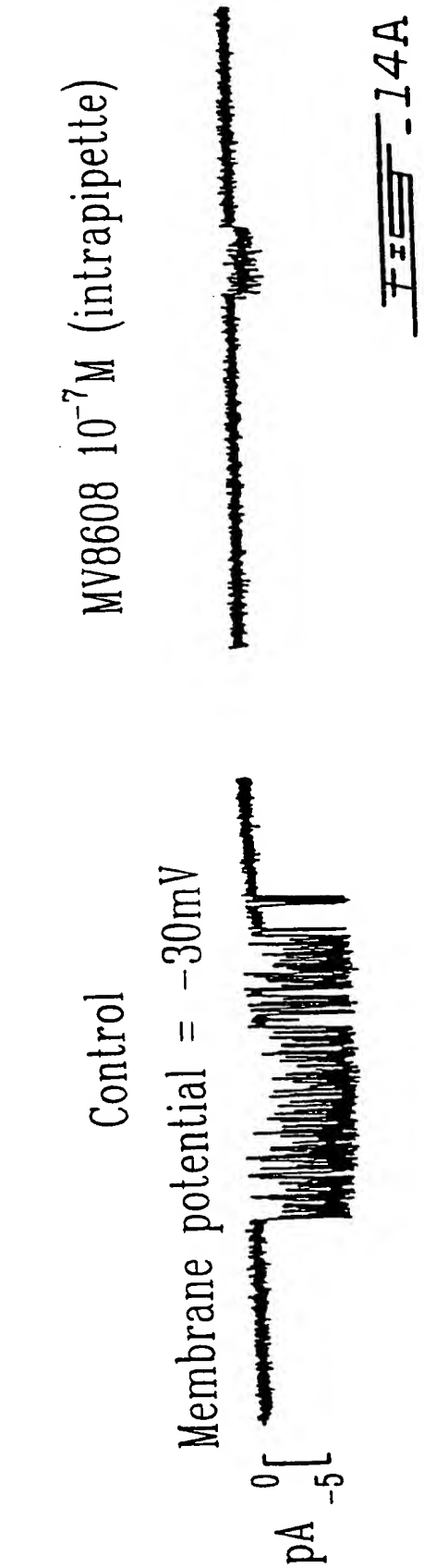


FIG. 14A

FIG. 14B

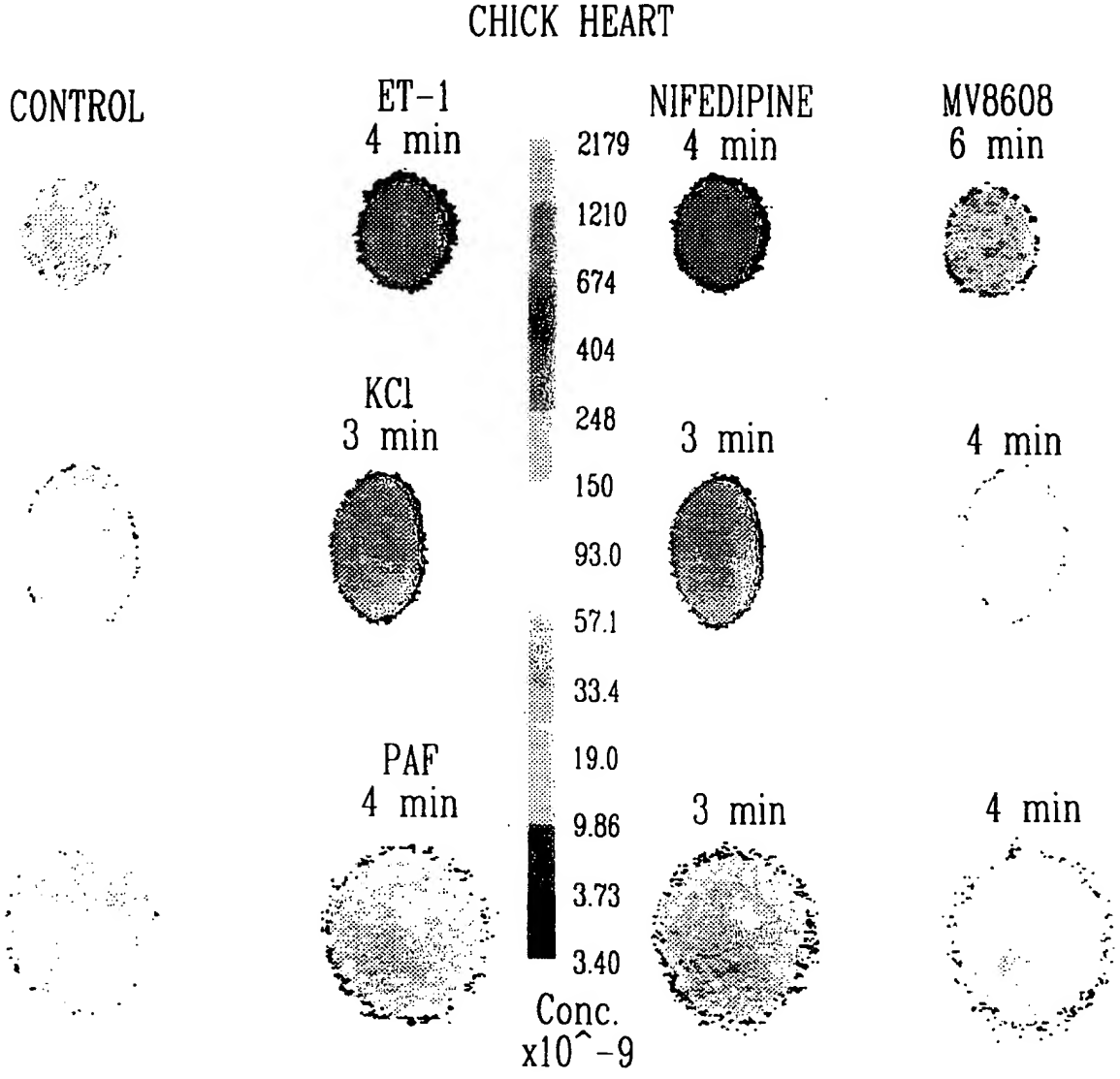

CHICK HEART
CONTROL
ET-1
4 min
NIFEDIPINE
4 min
MV8608
6 min
KCl
3 min
3 min
4 min
PAF
4 min
3 min
4 min
2179
1210
674
404
248
150
93.0
57.1
33.4
19.0
9.86
3.73
3.40
Conc.
x10^-9

Fig. 16

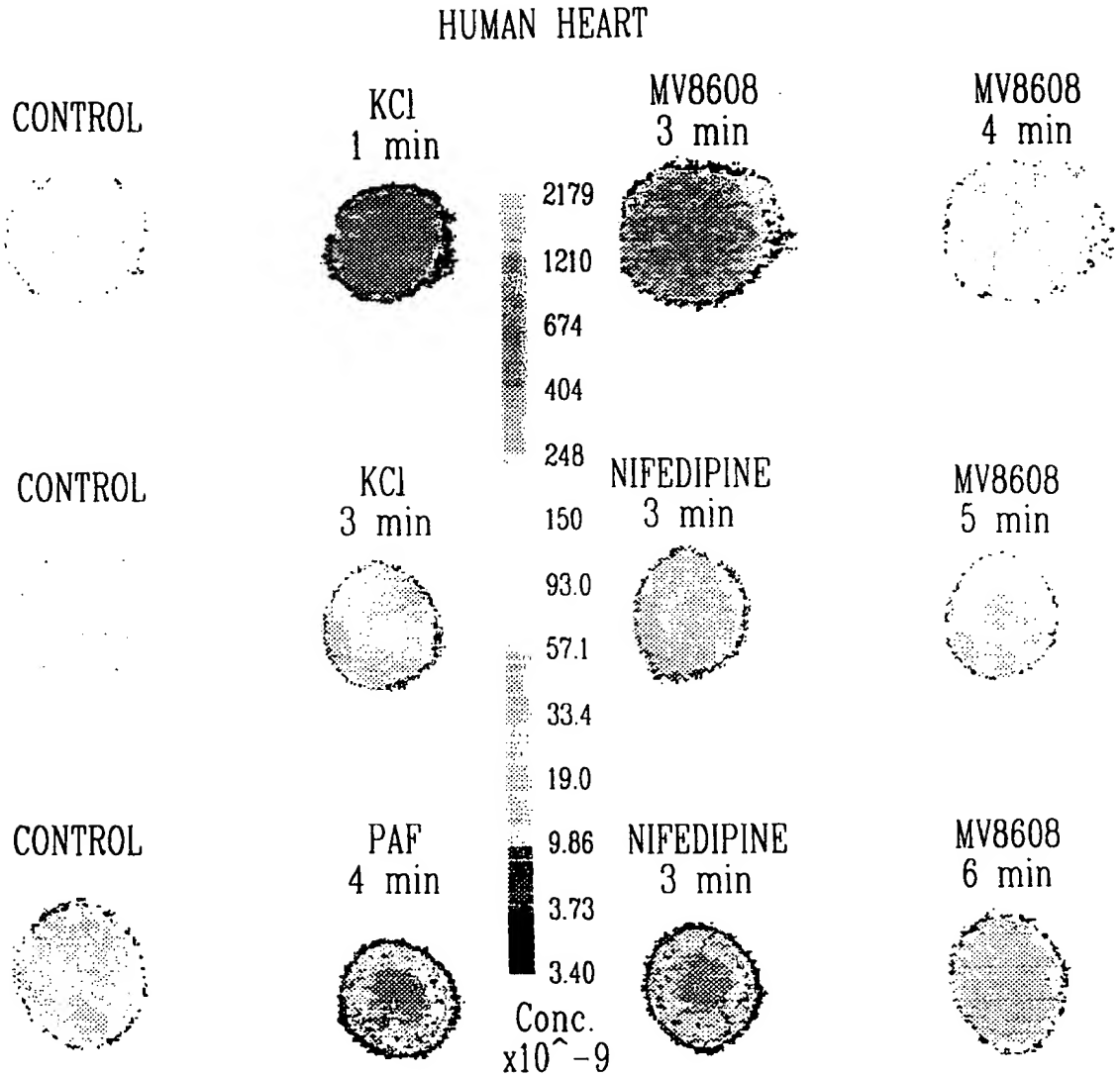

HUMAN HEART
CONTROL
KCl
1 min
MV8608
3 min
MV8608
4 min
2179
1210
674
404
248
CONTROL
KCl
3 min
NIFEDIPINE
3 min
MV8608
5 min
150
93.0
57.1
33.4
19.0
CONTROL
PAF
4 min
NIFEDIPINE
3 min
MV8608
6 min
9.86
3.73
3.40
Conc.
x10^-9

Fig. 17

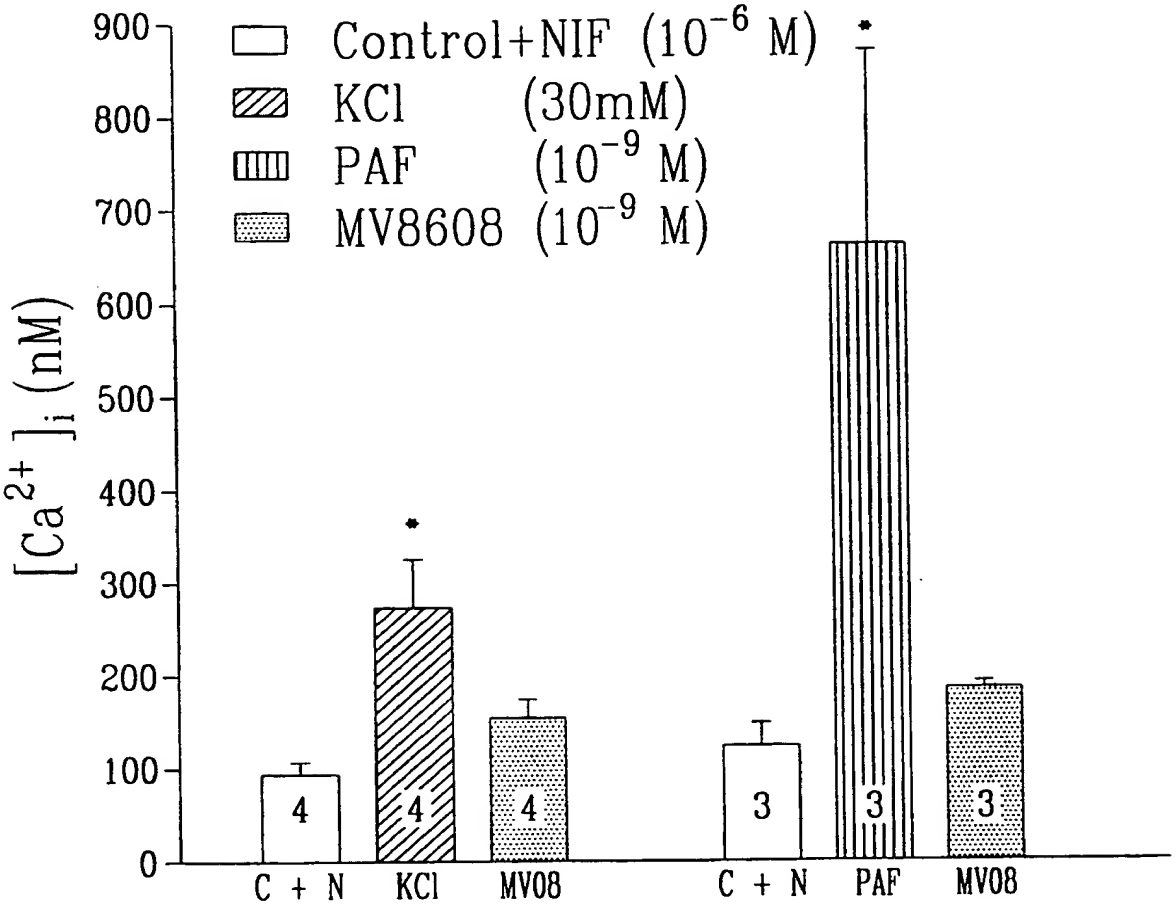

Control+NIF (10^{-6} M)
KCl (30mM)
PAF (10^{-9} M)
MV8608 (10^{-9} M)
$[Ca^{2+}]_i$ (nM)
900
800
700
600
500
400
300
200
100
0
4
4
4
3
3
3
C + N
KCl
MV08
C + N
PAF
MV08

Fig - 1B

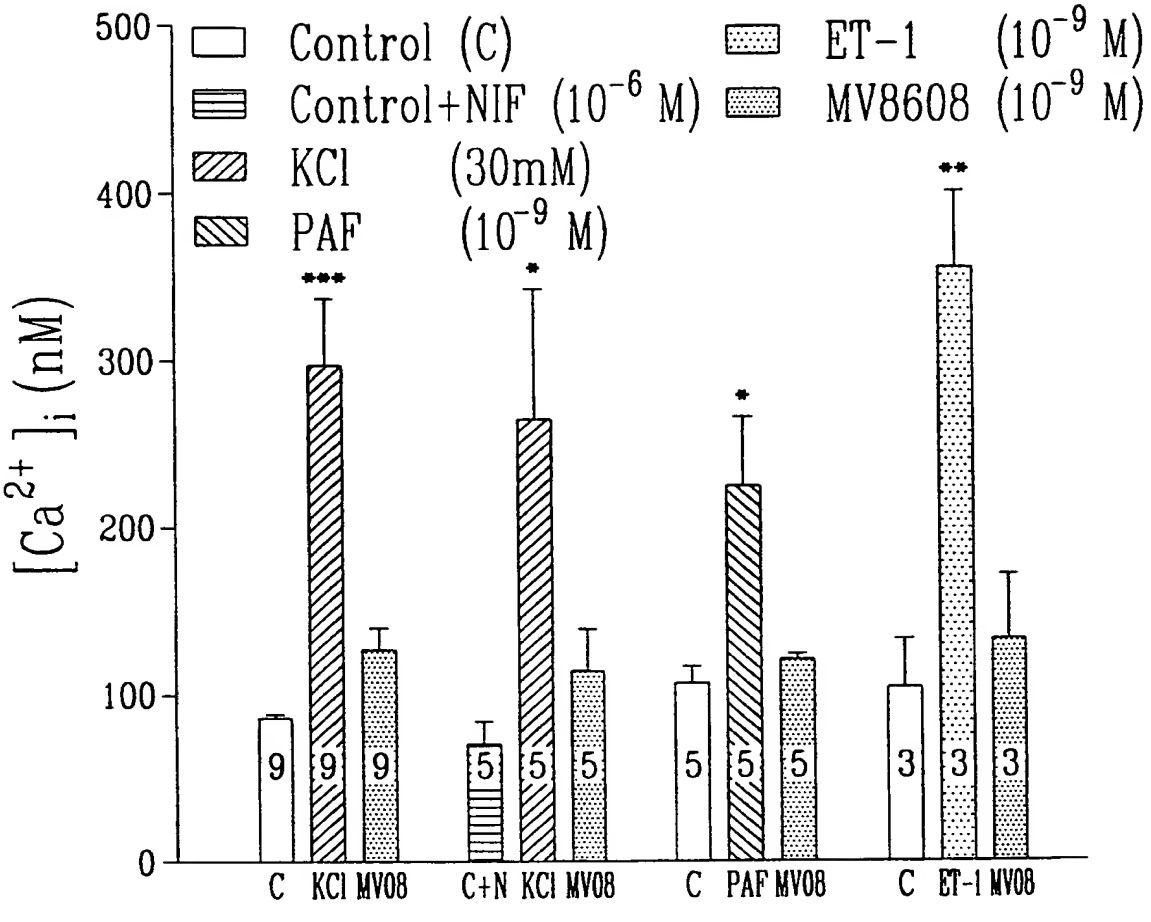

Control (C)
Control+NIF (10^{-6} M)
KCl (30mM)
PAF (10^{-9} M)
ET-1 (10^{-9} M)
MV8608 (10^{-9} M)
$[Ca^{2+}]_i$ (nM)
500
400
300
200
100
0
***
*
*
**
9
9
9
5
5
5
5
5
5
3
3
3
C KCl MV08 C+N KCl MV08 C PAF MV08 C ET-1 MV08

Fig. 19

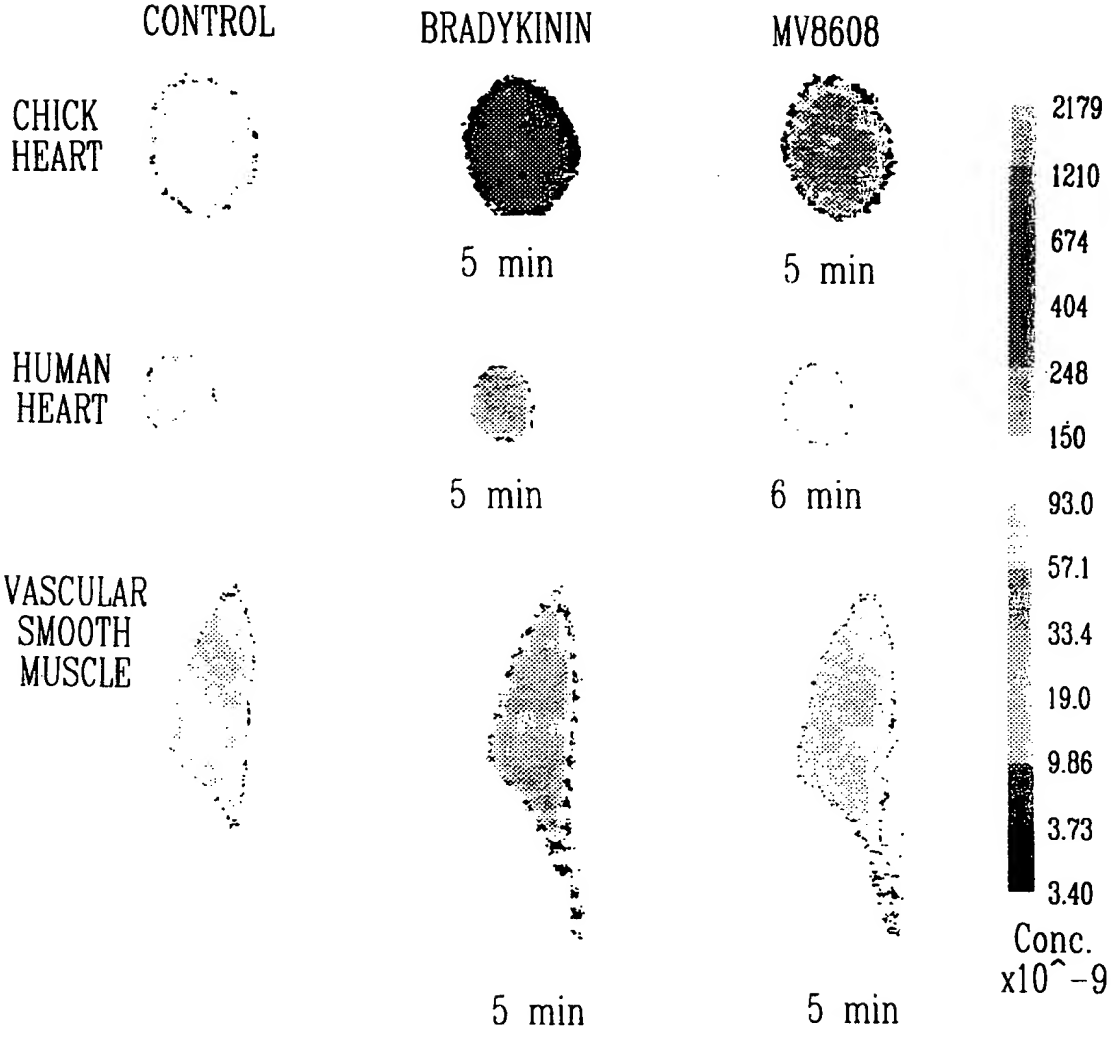

CONTROL
BRADYKININ
MV8608
CHICK HEART
5 min
5 min
HUMAN HEART
5 min
6 min
VASCULAR SMOOTH MUSCLE
5 min
5 min
2179
1210
674
404
248
150
93.0
57.1
33.4
19.0
9.86
3.73
3.40
Conc. x10^-9

FIG. 20

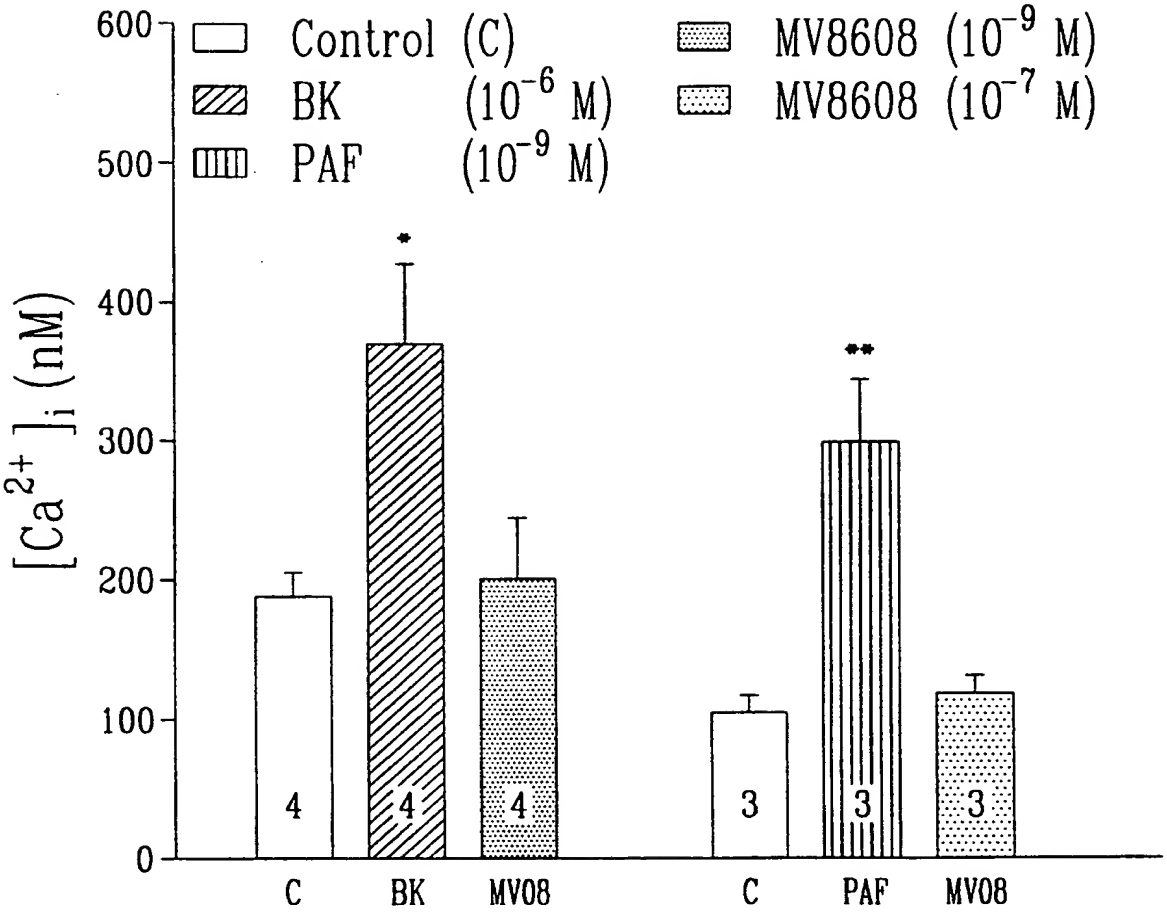

600
500
400
300
200
100
0
$[Ca^{2+}]_i$ (nM)
Control (C)
BK (10^{-6} M)
PAF (10^{-9} M)
MV8608 (10^{-9} M)
MV8608 (10^{-7} M)
*
**
4
4
4
3
3
3
C
BK
MV08
C
PAF
MV08

Fig. 21

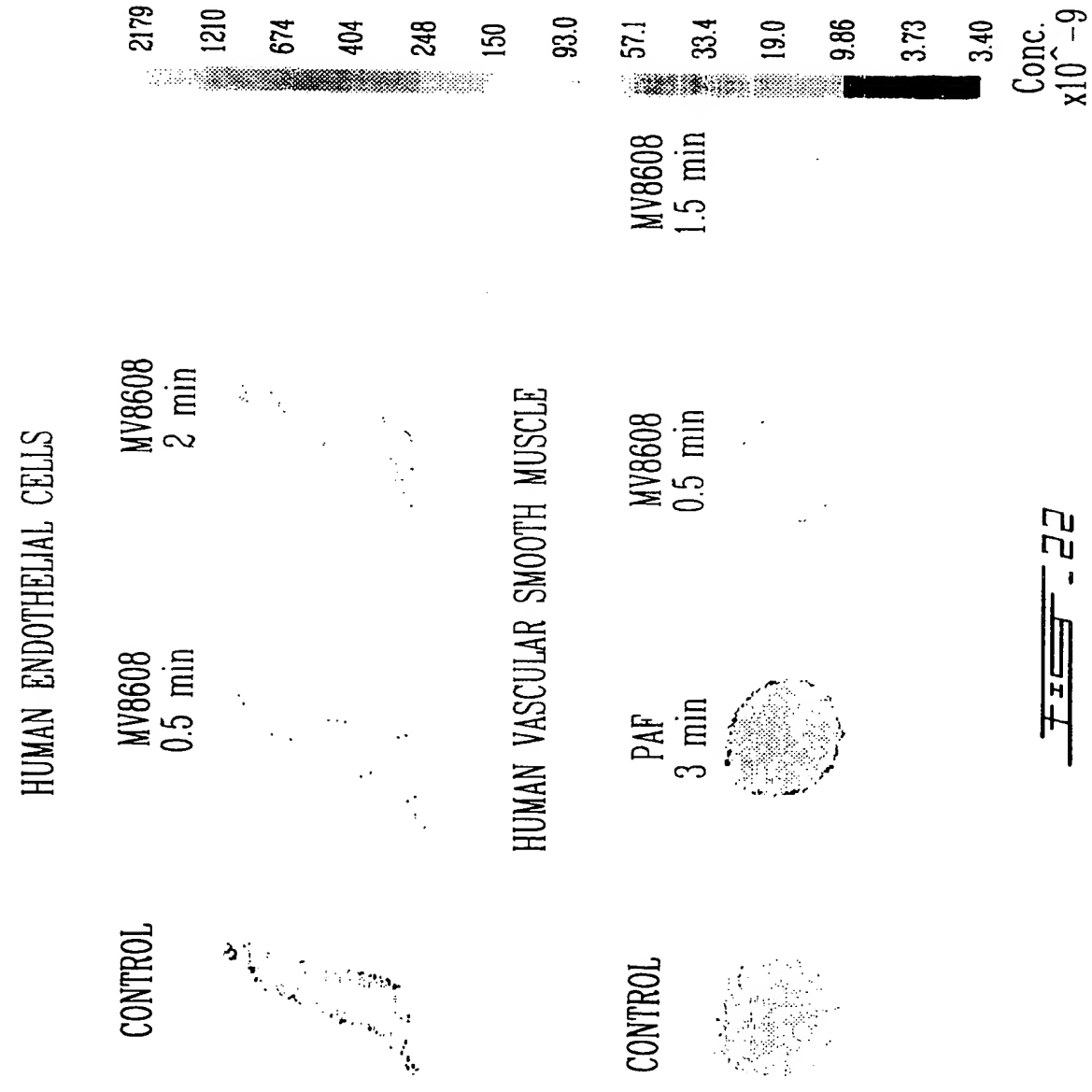

HUMAN ENDOTHELIAL CELLS
CONTROL
MV8608 0.5 min
MV8608 2 min
HUMAN VASCULAR SMOOTH MUSCLE
CONTROL
PAF 3 min
MV8608 0.5 min
MV8608 1.5 min
2179
1210
674
404
248
150
93.0
57.1
33.4
19.0
9.86
3.73
3.40
Conc. x10^-9

Fig. 22

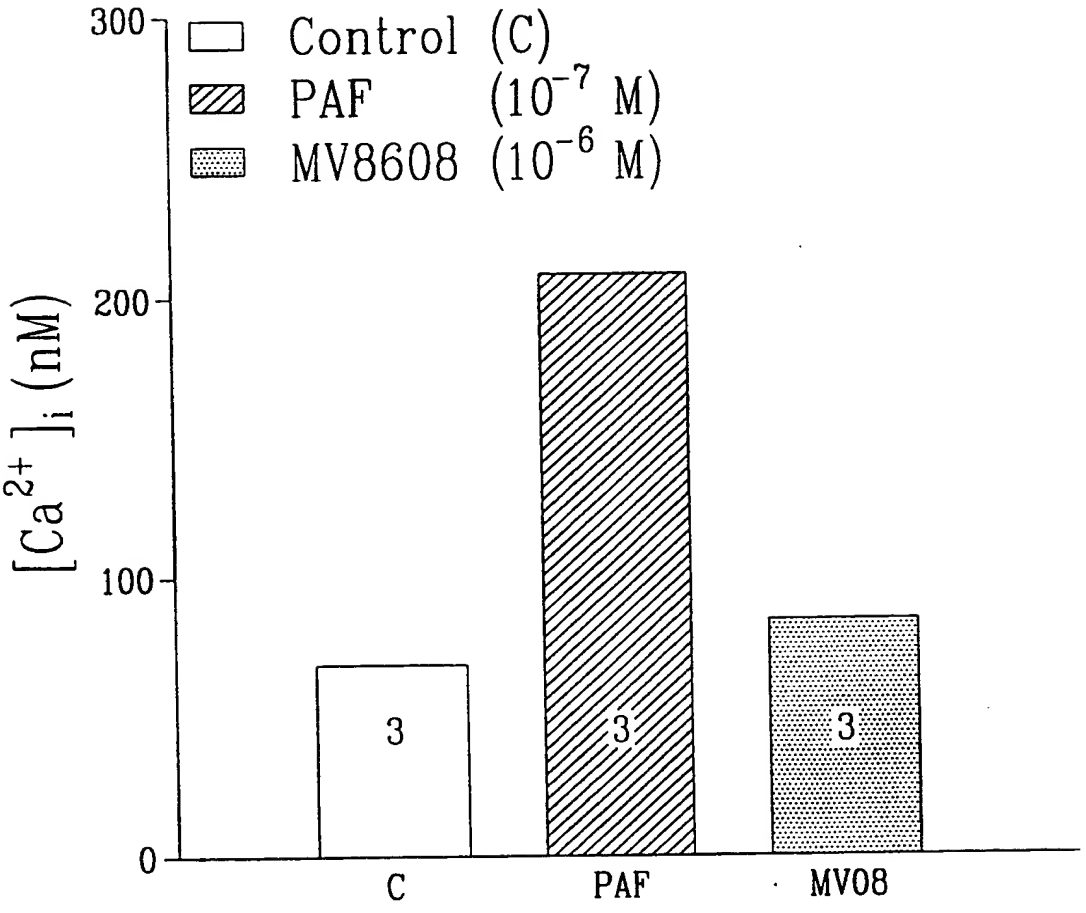

300
200
100
0
Control (C)
PAF (10^{-7} M)
MV8608 (10^{-6} M)
$[Ca^{2+}]_i$ (nM)
3
3
3
C
PAF
MV08

Fig 23

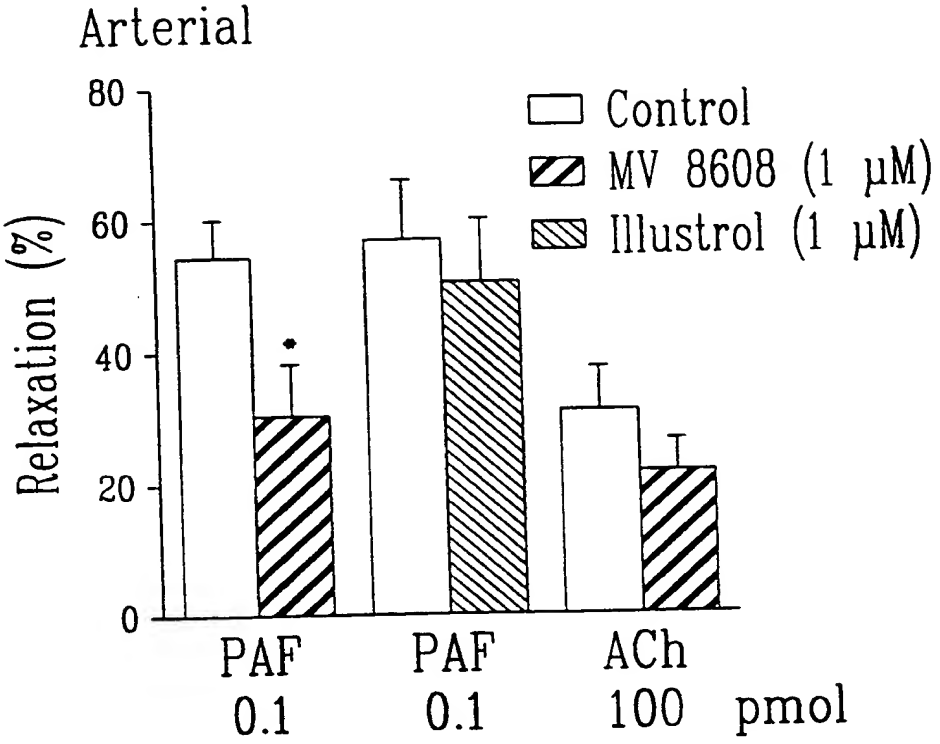


FIG. 24A

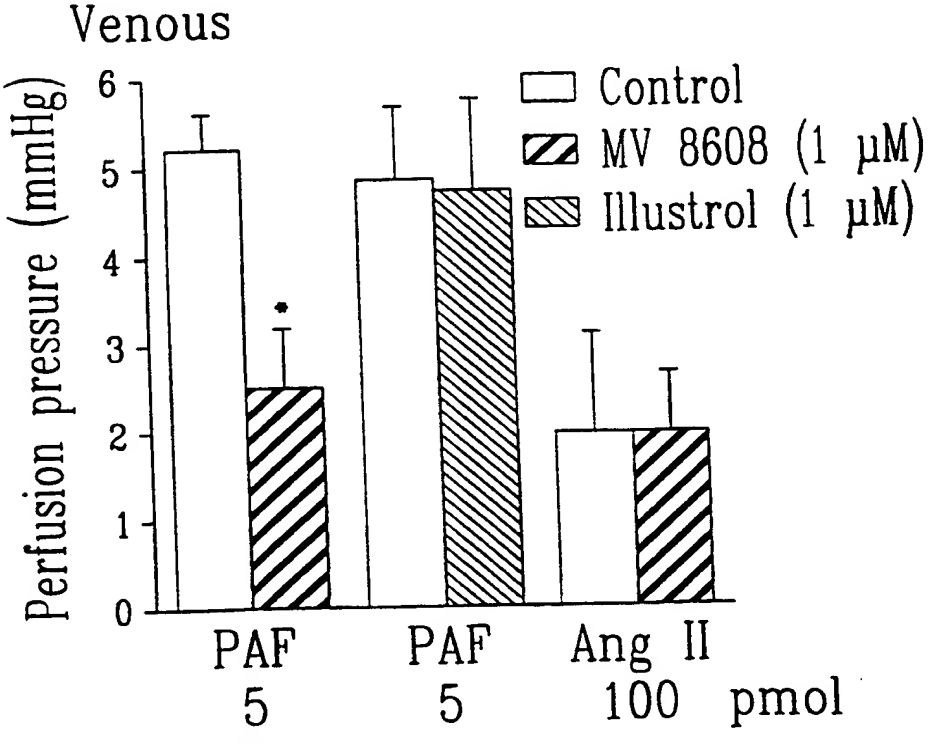


FIG. 24B

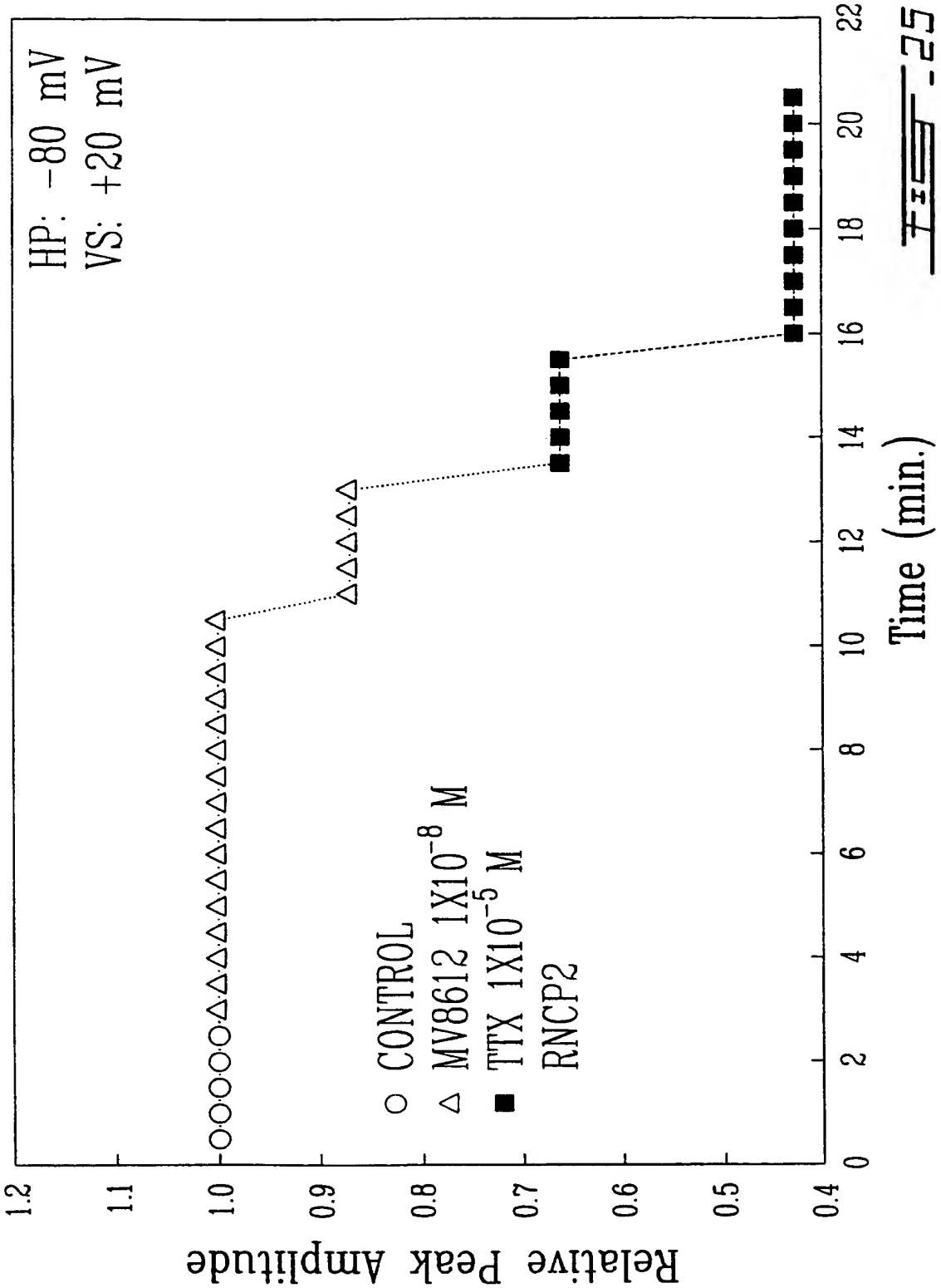

HP: −80 mV
VS: +20 mV
○ CONTROL
△ MV8612 1X10^{-8} M
■ TTX 1X10^{-5} M
RNCP2
Relative Peak Amplitude
Time (min.)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
2
4
6
8
10
12
14
16
18
20
22

Fig -25

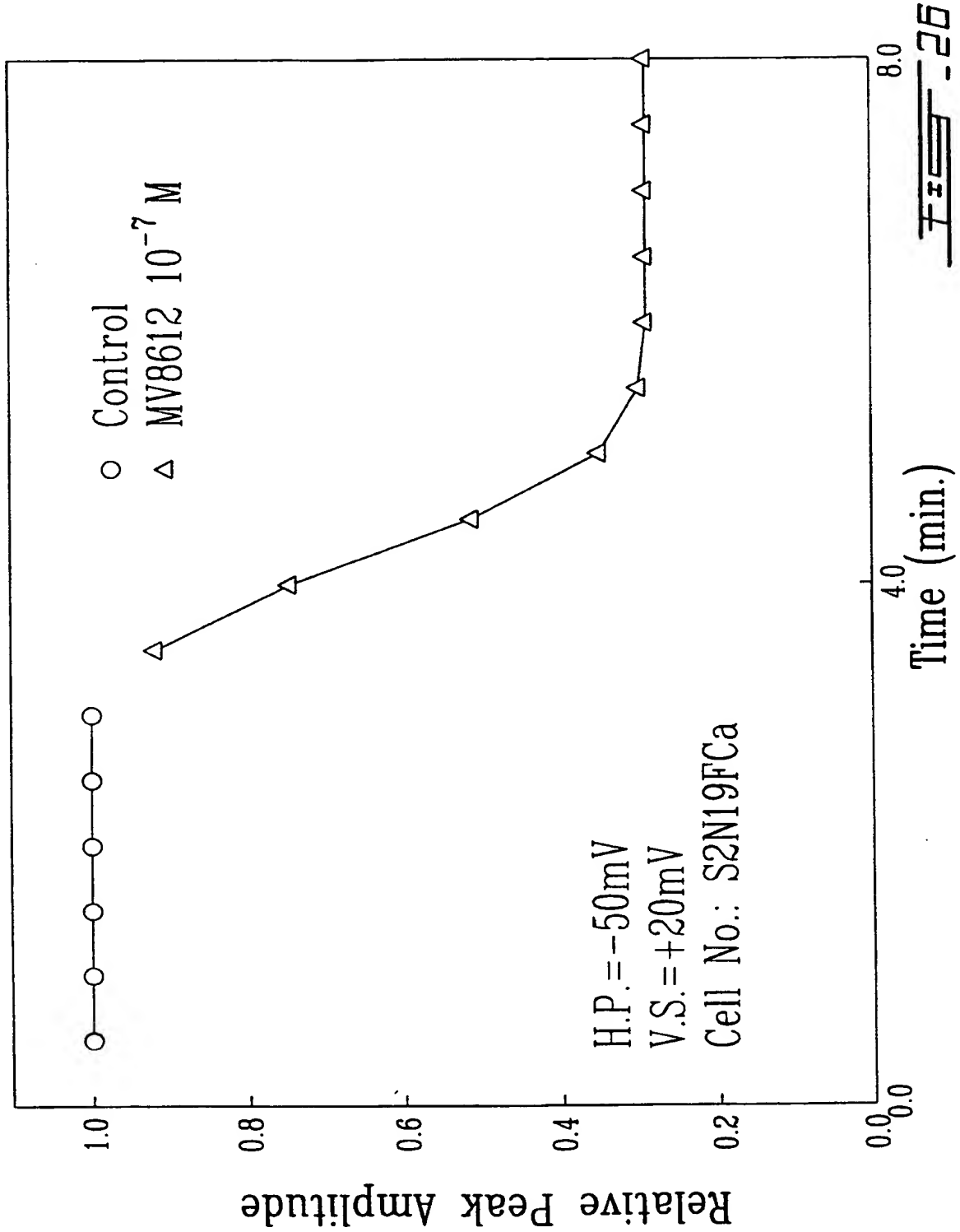

Relative Peak Amplitude
1.0
0.8
0.6
0.4
0.2
0.0
0.0
4.0
8.0
Time (min.)
○ Control
△ MV8612 10^{-7} M
H.P.=−50mV
V.S.=+20mV
Cell No.: S2N19FCa

Fig-26

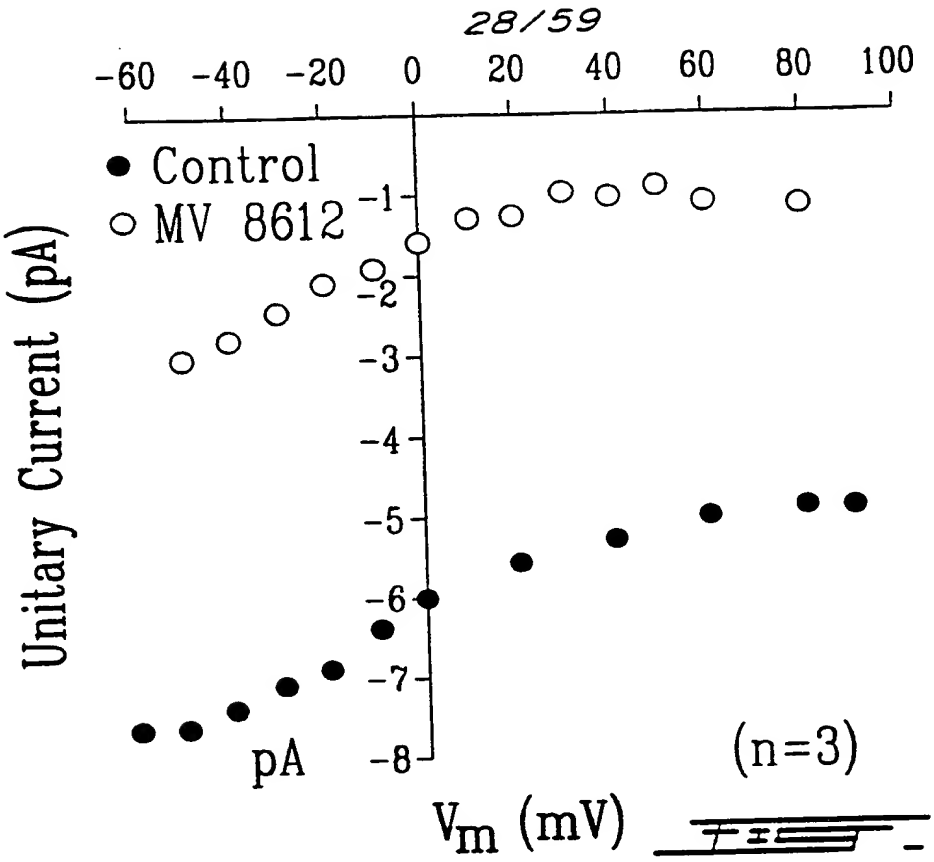


Fig 27A

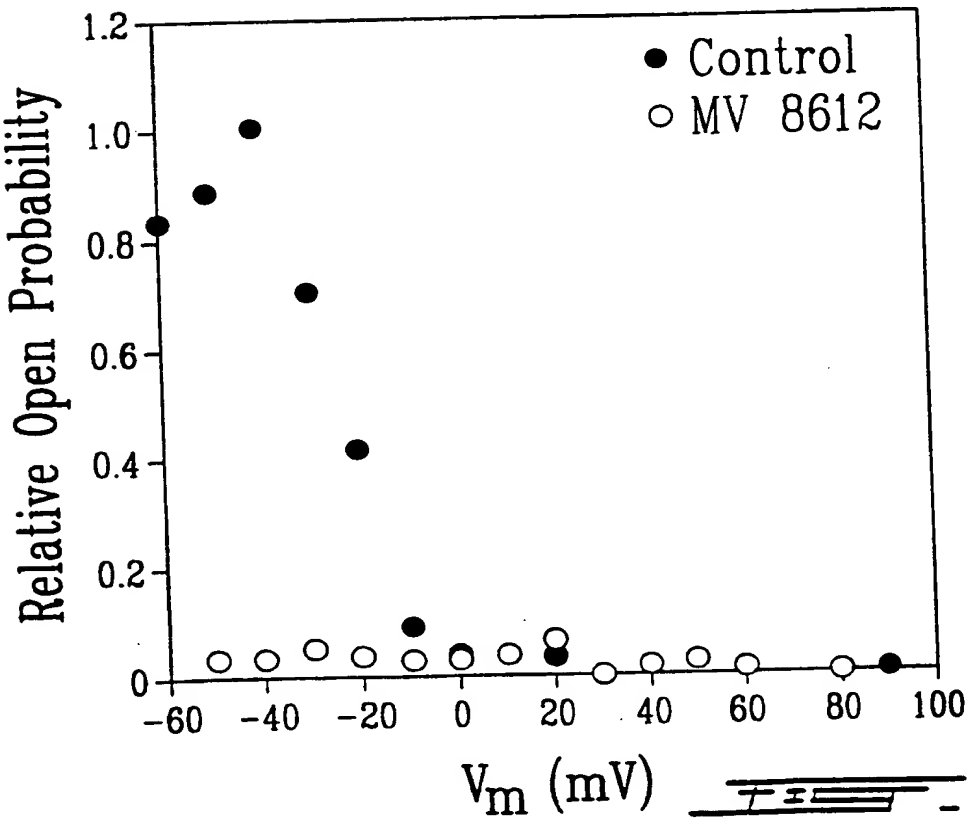
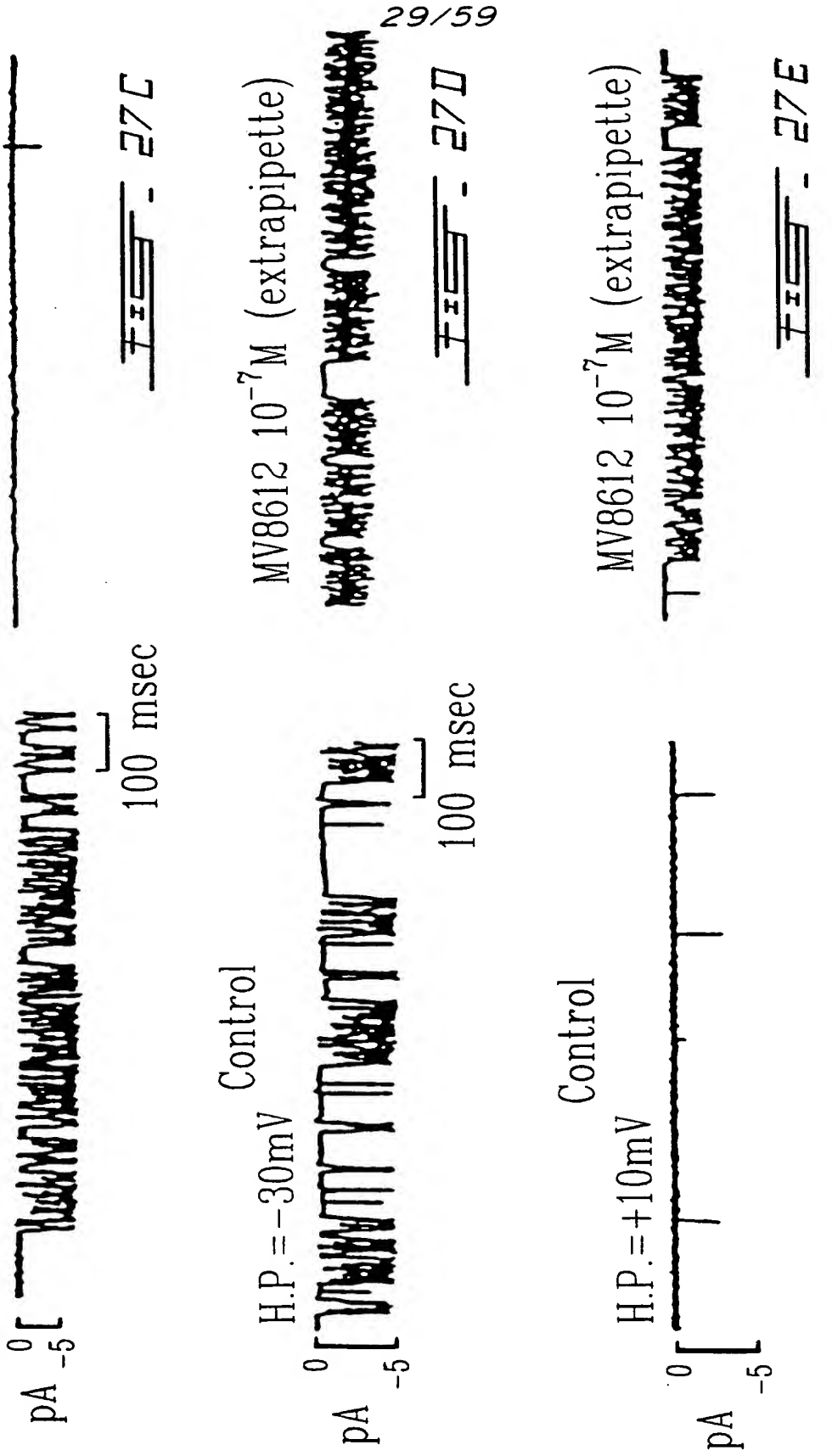
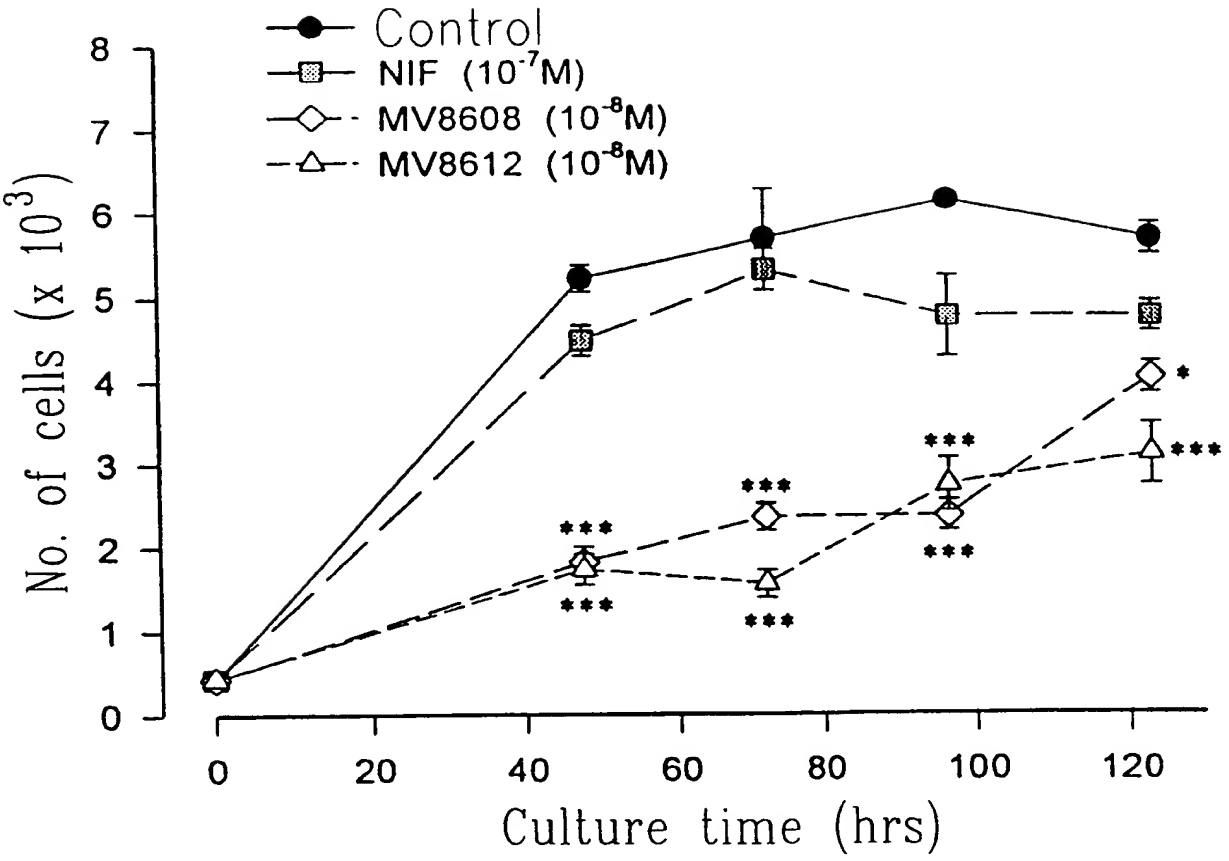


Fig 27B


Control
H.P.=−30mV
pA 0 −5
100 msec
MV8612 10⁻⁷M (intrapipette)
27C
Control
H.P.=−30mV
pA 0 −5
100 msec
MV8612 10⁻⁷M (extrapipette)
27D
Control
H.P.=+10mV
pA 0 −5
MV8612 10⁻⁷M (extrapipette)
27E



* $p < 0.05$ *** $p < 0.001$

Fig 28

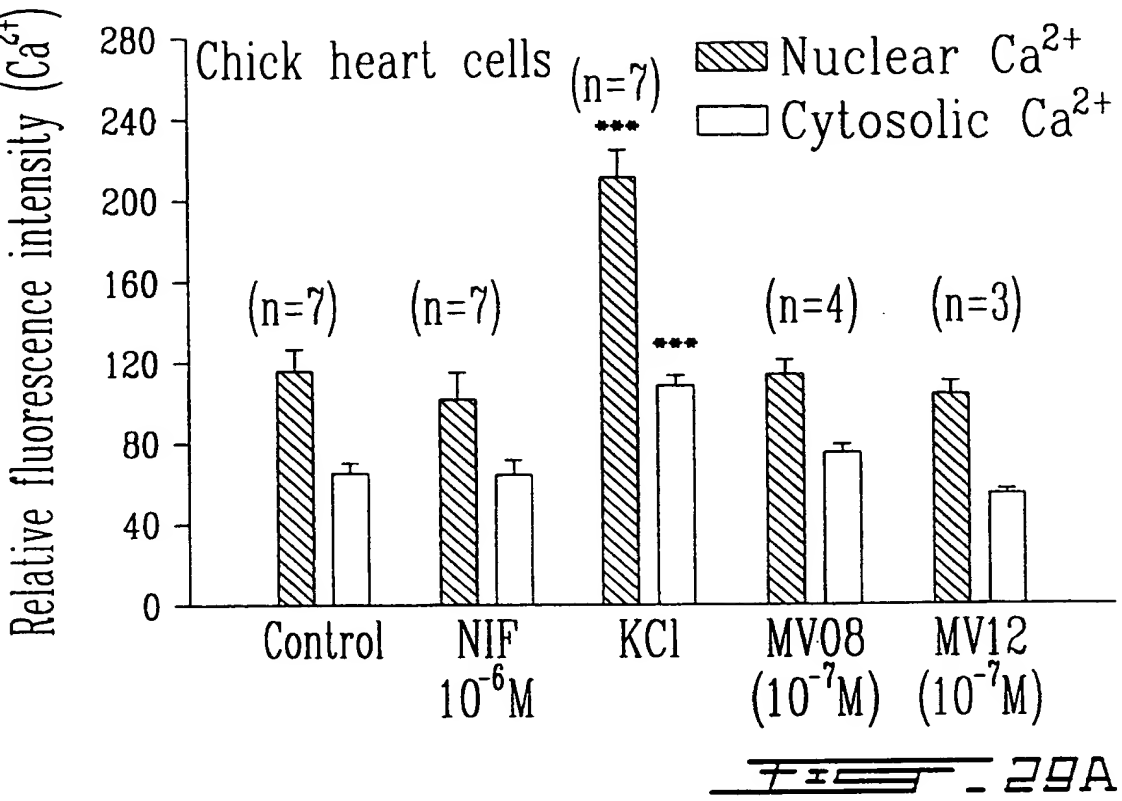


Fig. 29A

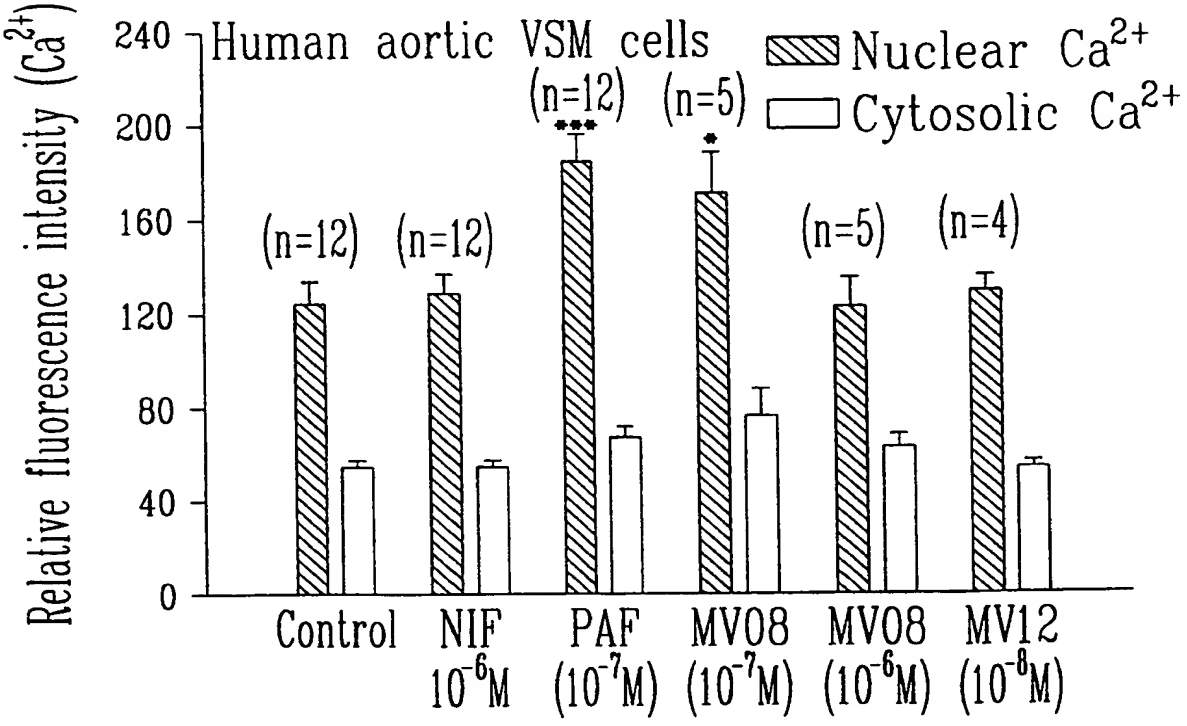


Fig. 29B

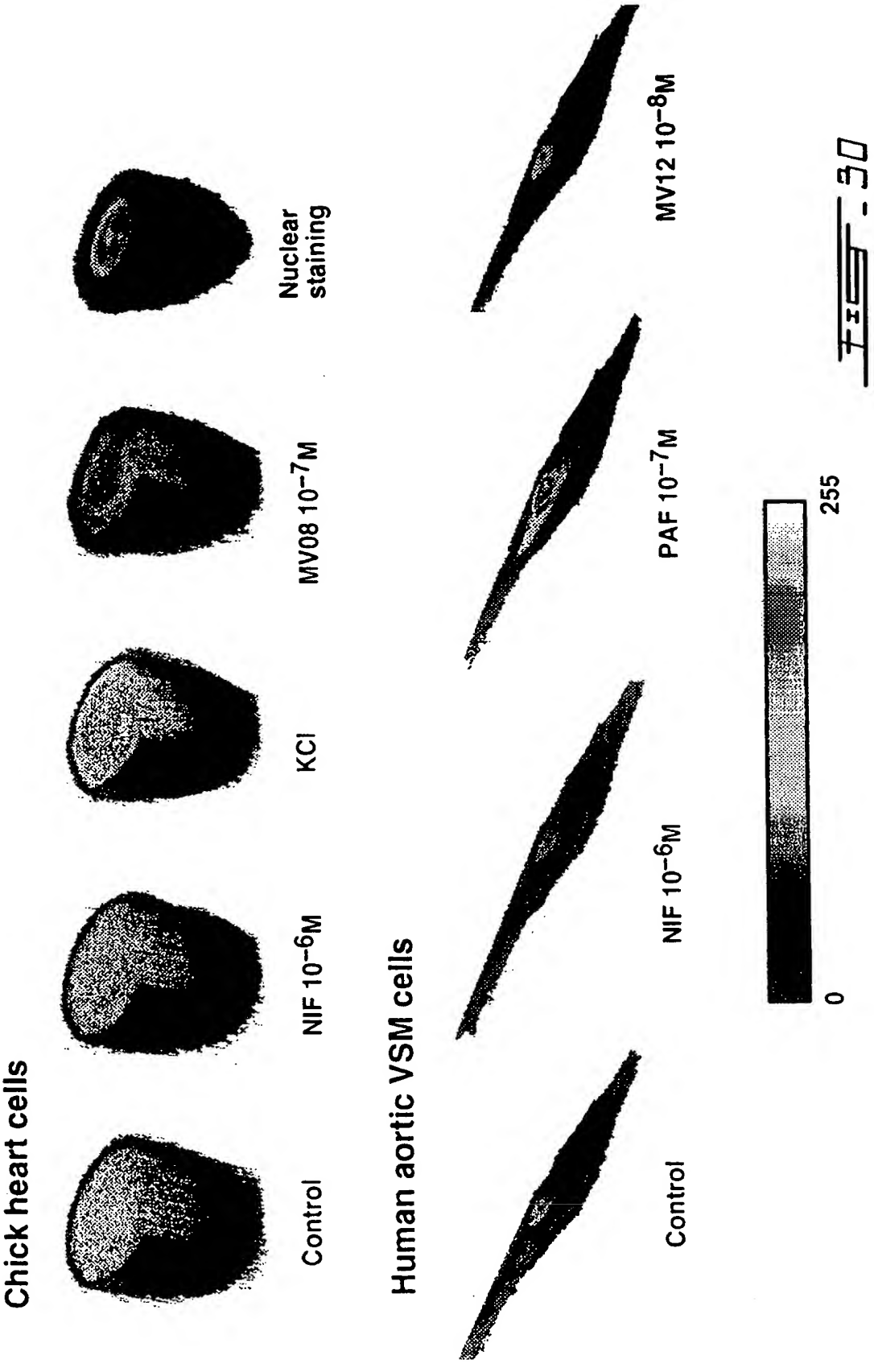

Chick heart cells
Control
NIF 10⁻⁶M
KCl
MV08 10⁻⁷M
Nuclear staining
Human aortic VSM cells
Control
NIF 10⁻⁶M
PAF 10⁻⁷M
MV12 10⁻⁸M
255
0

Fig - 30

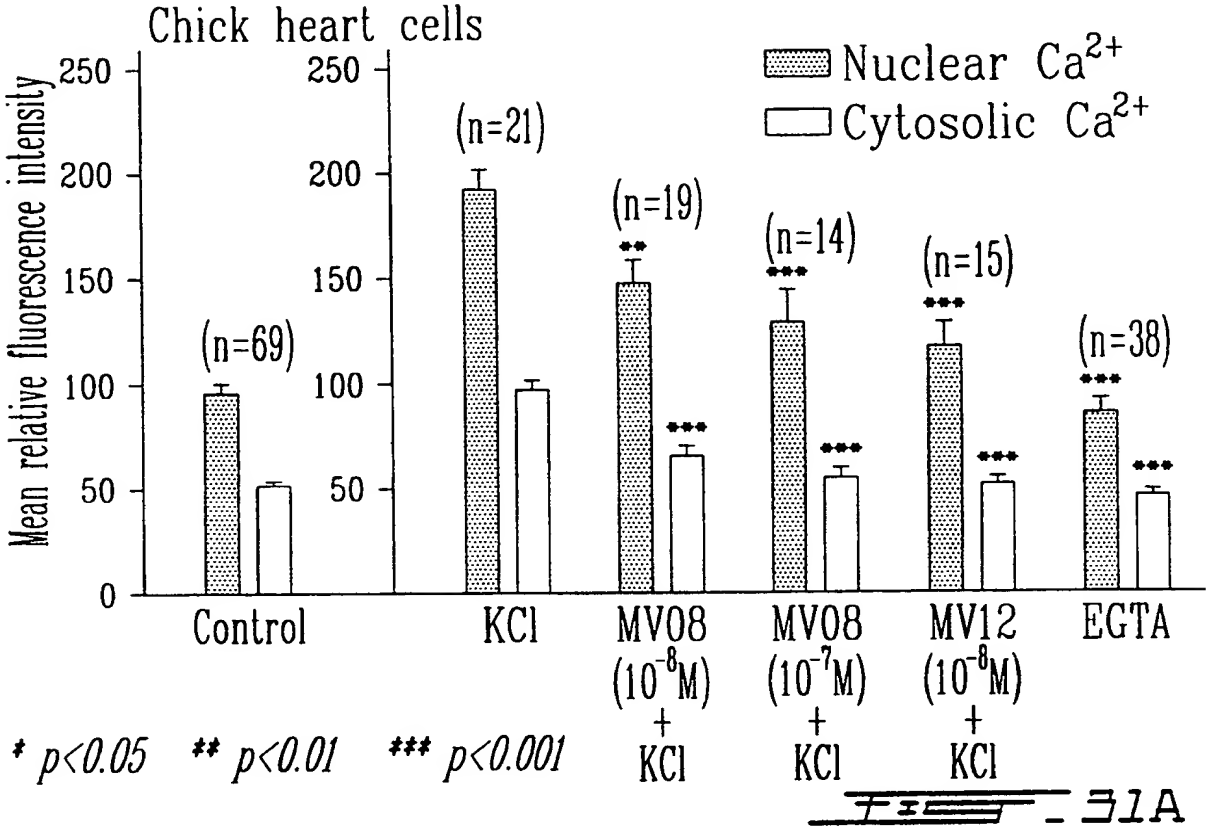


Fig 31A

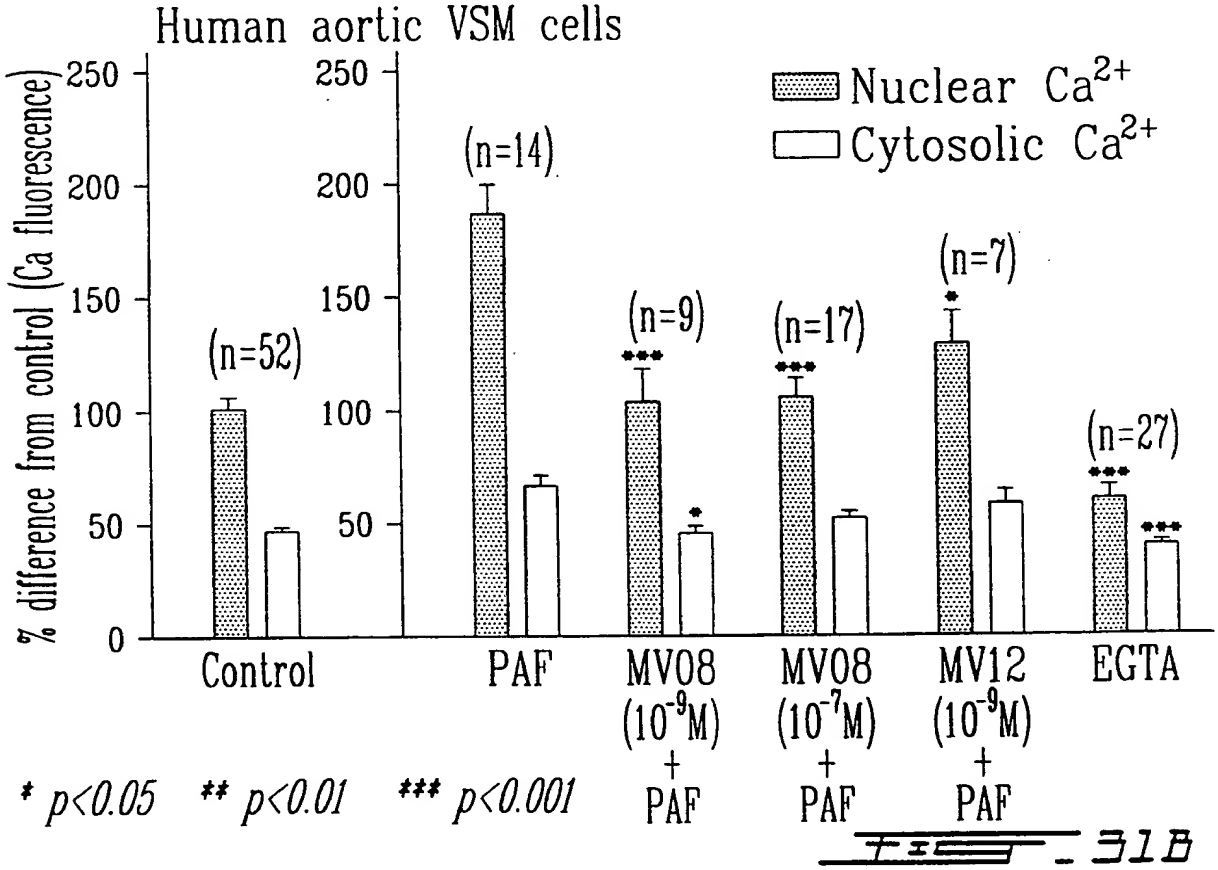


Fig 31B

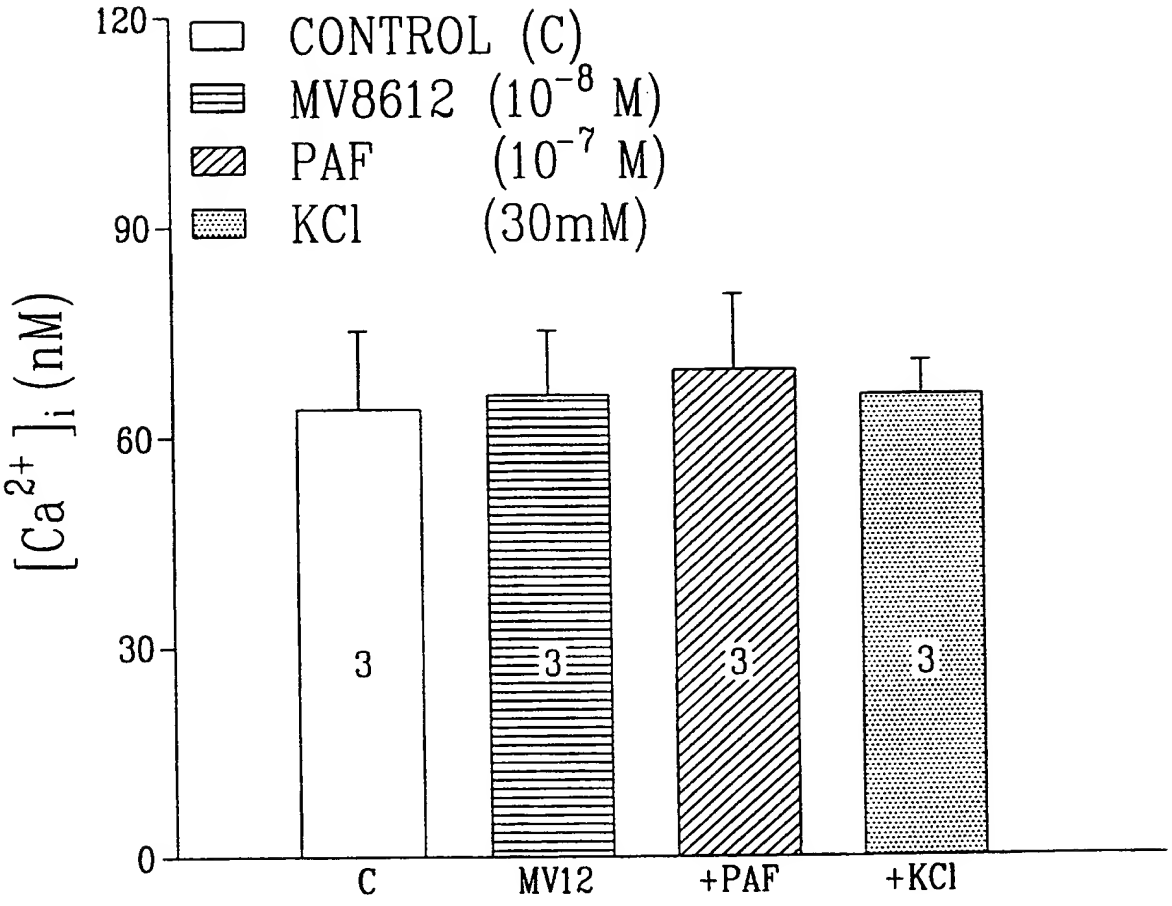

120
90
60
30
0
CONTROL (C)
MV8612 (10^{-8} M)
PAF (10^{-7} M)
KCl (30mM)
$[Ca^{2+}]_i$ (nM)
3
3
3
3
C
MV12
+PAF
+KCl

Fig - 32

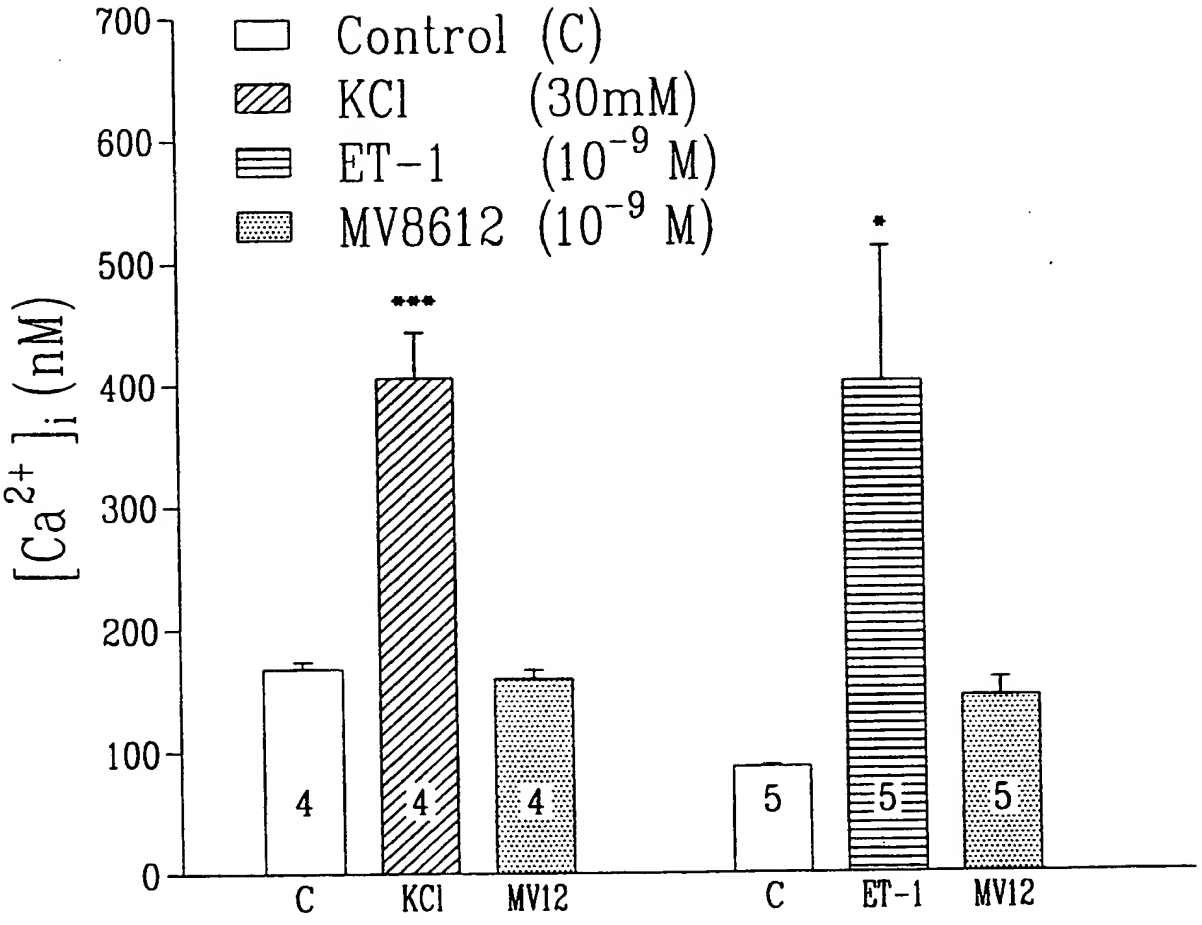

Control (C)
KCl (30mM)
ET-1 (10^{-9} M)
MV8612 (10^{-9} M)
$[Ca^{2+}]_i$ (nM)
700
600
500
400
300
200
100
0
***
*
4
4
4
5
5
5
C
KCl
MV12
C
ET-1
MV12

Fig. 33

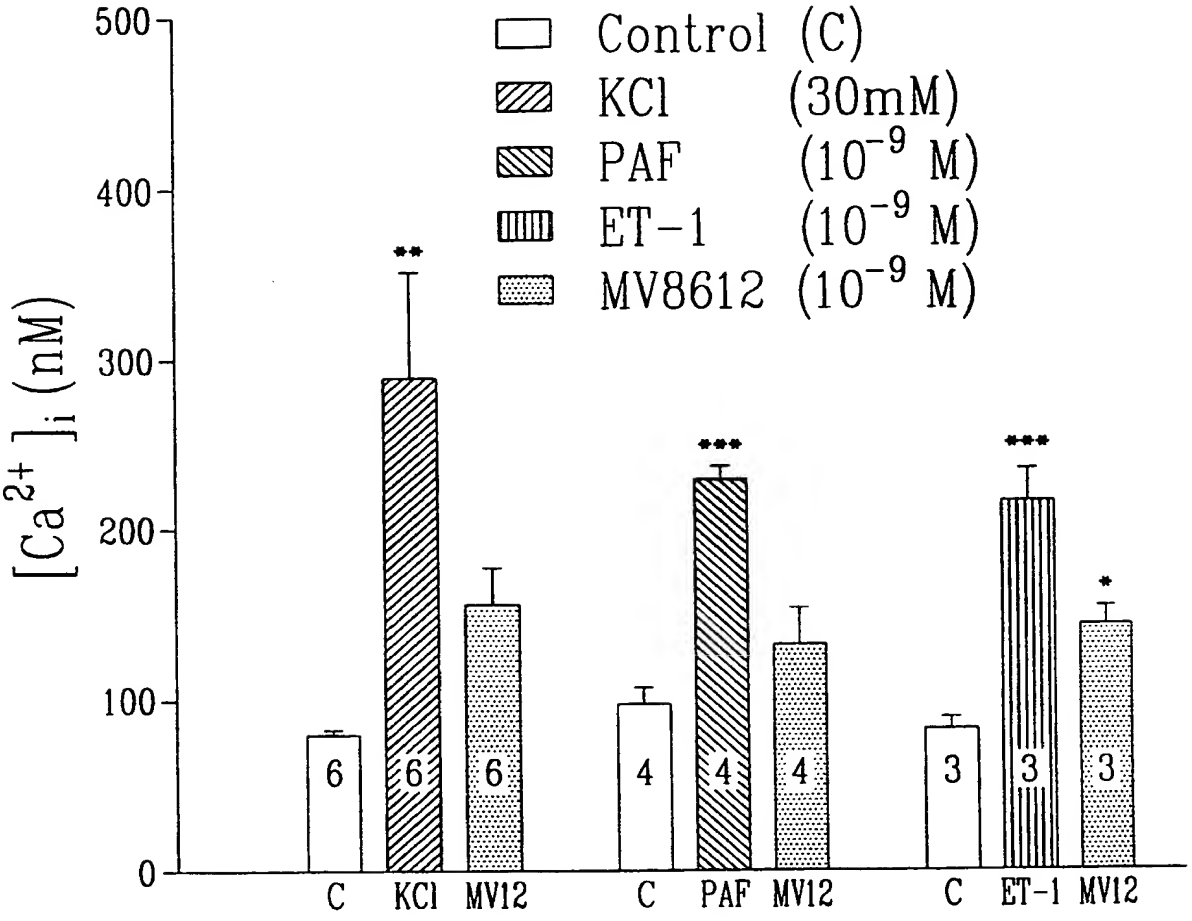

Control (C)
KCl (30mM)
PAF (10^{-9} M)
ET-1 (10^{-9} M)
MV8612 (10^{-9} M)
$[Ca^{2+}]_i$ (nM)
500
400
300
200
100
0
**
***
***
*
6
6
6
4
4
4
3
3
3
C
KCl
MV12
C
PAF
MV12
C
ET-1
MV12

Fig - 34

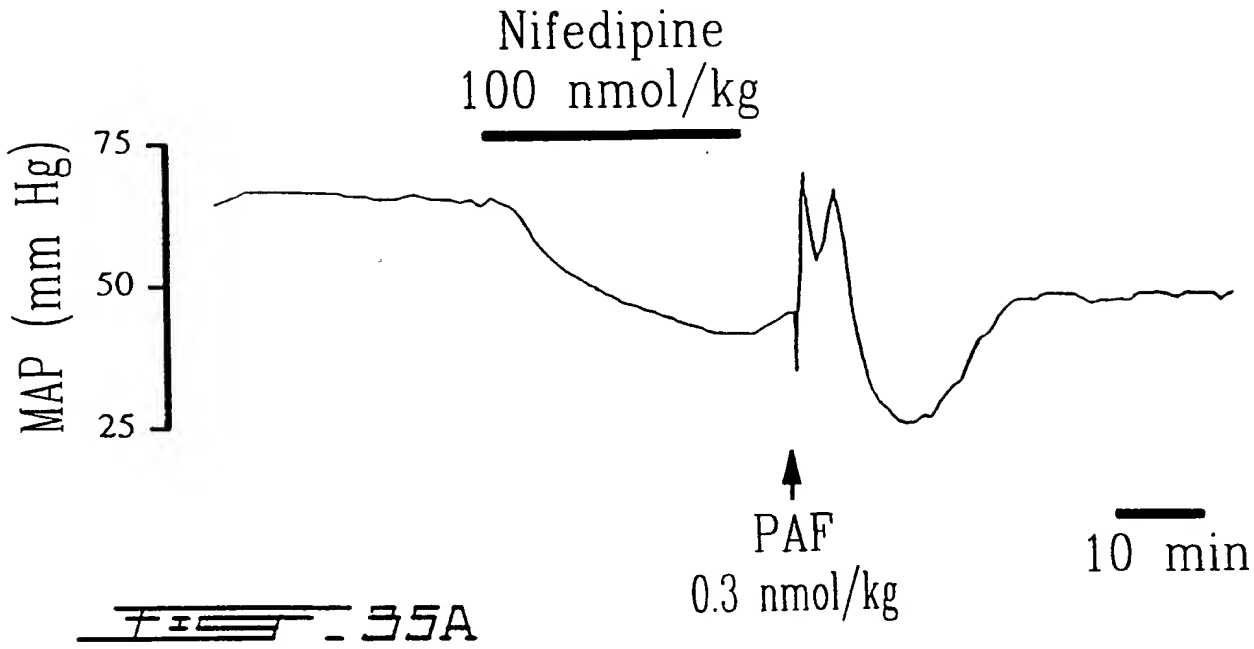


Fig. 35A

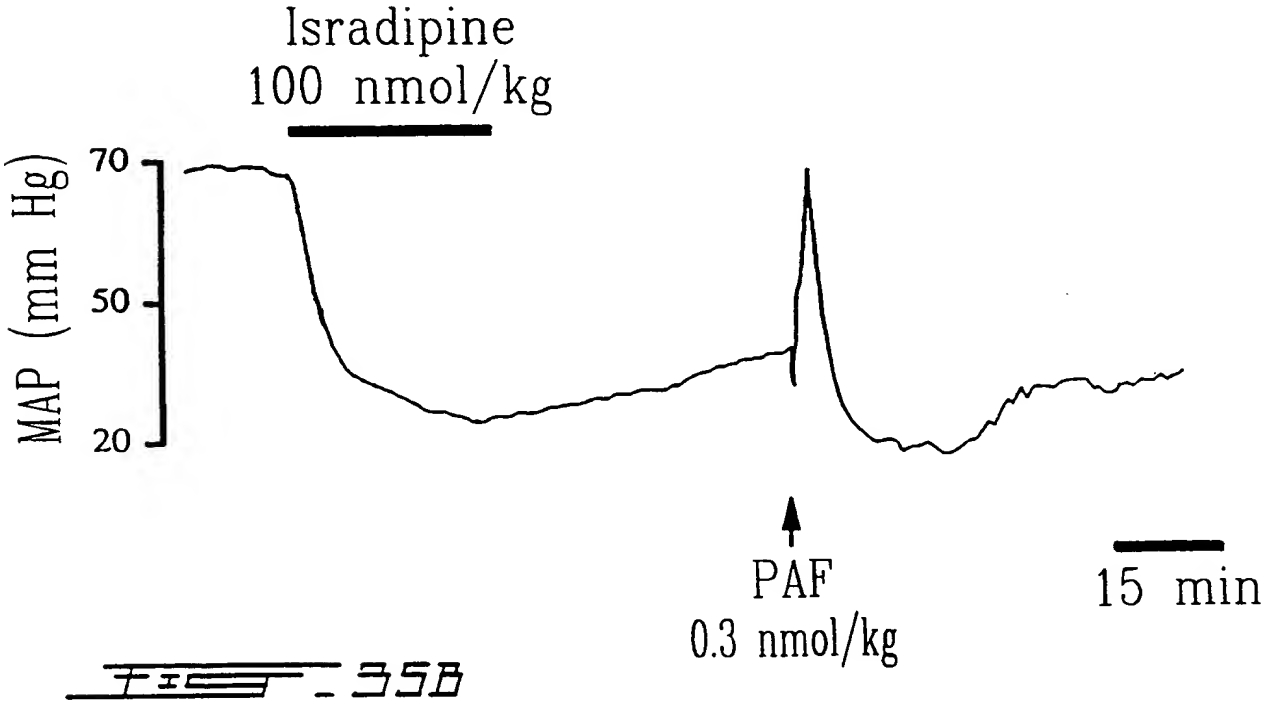


Fig. 35B

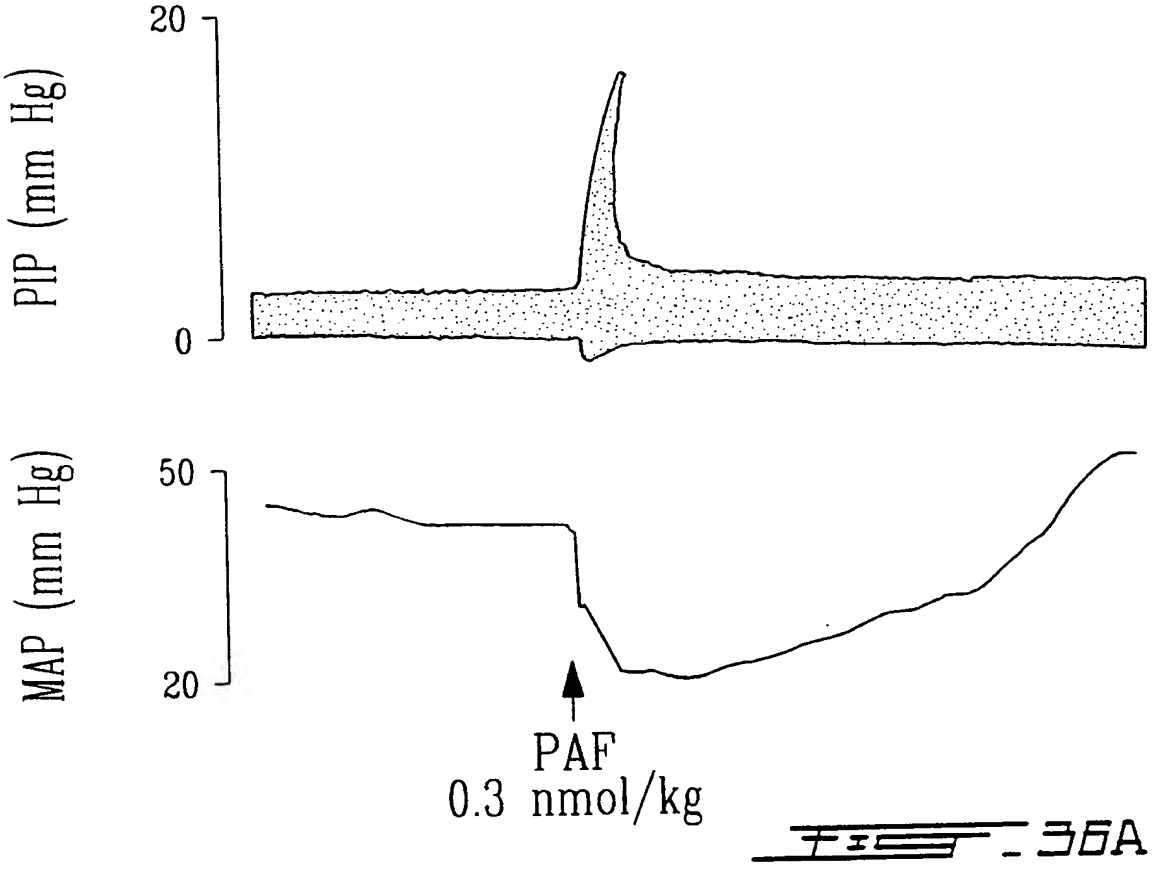


Fig 36A

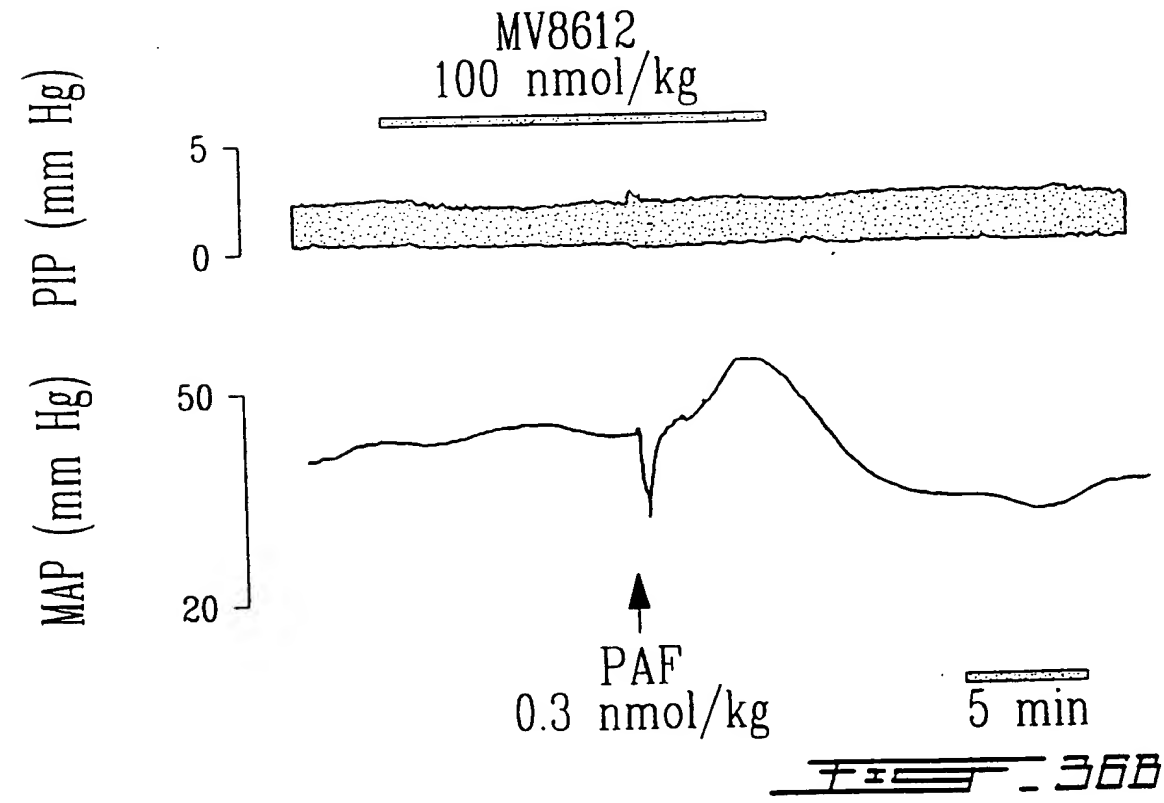
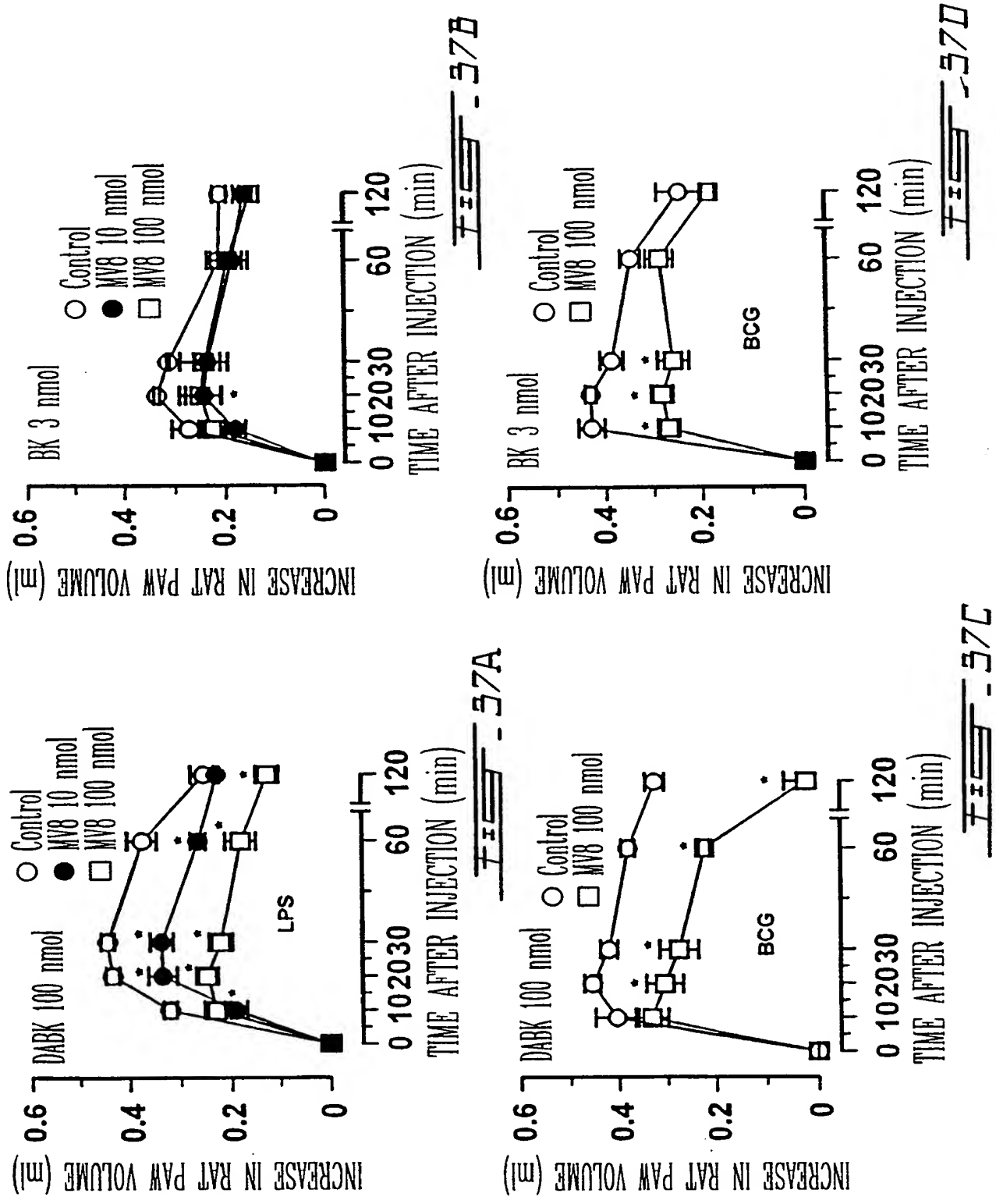
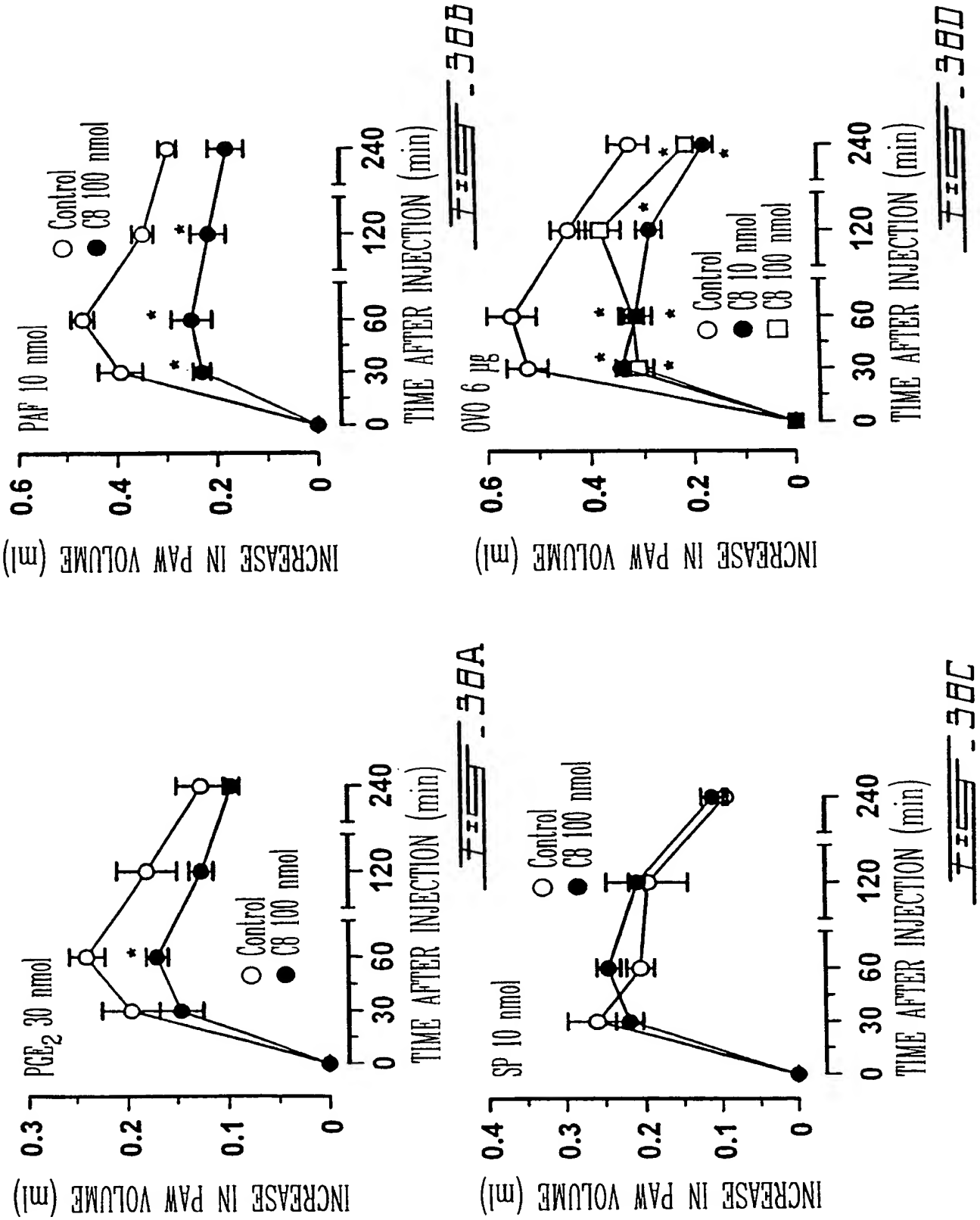


Fig 36B


INCREASE IN RAT PAW VOLUME (ml)
0.6
0.4
0.2
0
DABK 100 nmol
LPS
○ Control
● MV8 10 nmol
□ MV8 100 nmol
0 10 20 30 60 120
TIME AFTER INJECTION (min)
FIG - 37A
INCREASE IN RAT PAW VOLUME (ml)
0.6
0.4
0.2
0
BK 3 nmol
○ Control
● MV8 10 nmol
□ MV8 100 nmol
0 10 20 30 60 120
TIME AFTER INJECTION (min)
FIG - 37B
INCREASE IN RAT PAW VOLUME (ml)
0.6
0.4
0.2
0
DABK 100 nmol
BCG
○ Control
□ MV8 100 nmol
0 10 20 30 60 120
TIME AFTER INJECTION (min)
FIG - 37C
INCREASE IN RAT PAW VOLUME (ml)
0.6
0.4
0.2
0
BK 3 nmol
BCG
○ Control
□ MV8 100 nmol
0 10 20 30 60 120
TIME AFTER INJECTION (min)
FIG - 37D


PAF 10 nmol
OVO 6 µg
PGE2 30 nmol
SP 10 nmol
Control
C8 10 nmol
C8 100 nmol
INCREASE IN PAW VOLUME (ml)
TIME AFTER INJECTION (min)
0
30
60
120
240
0.1
0.2
0.3
0.4
0.6
FIG - 3BA
FIG - 3BB
FIG - 3BC
FIG - 3BD

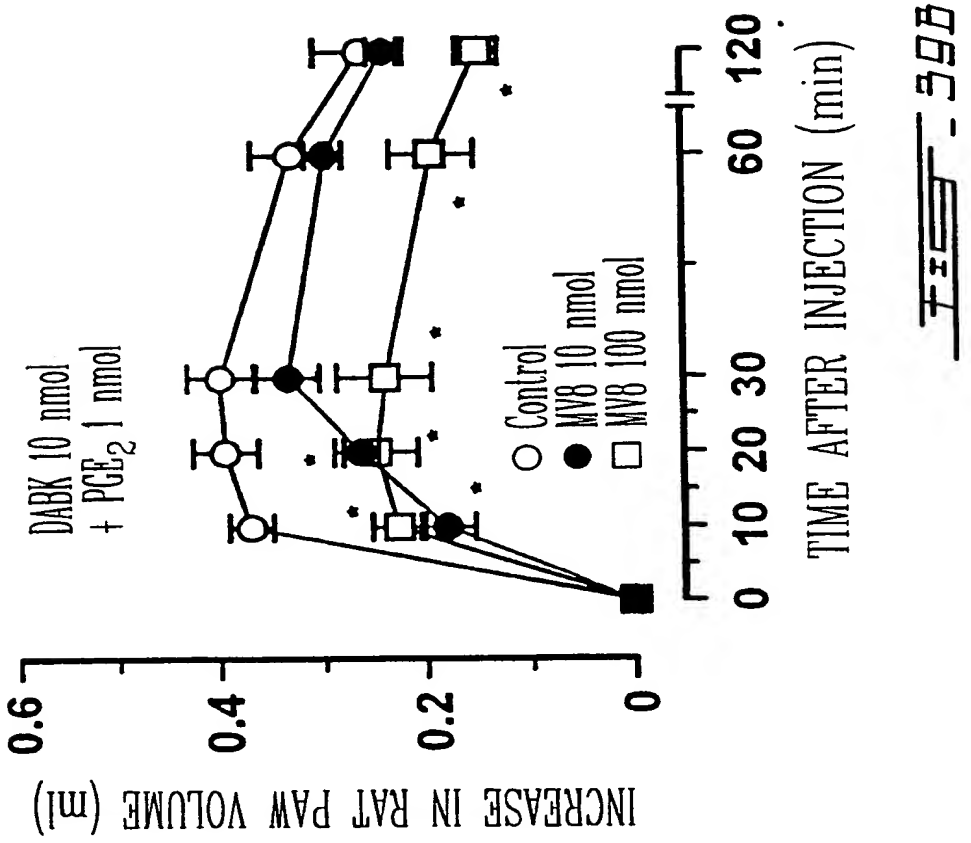


FIG - 39B

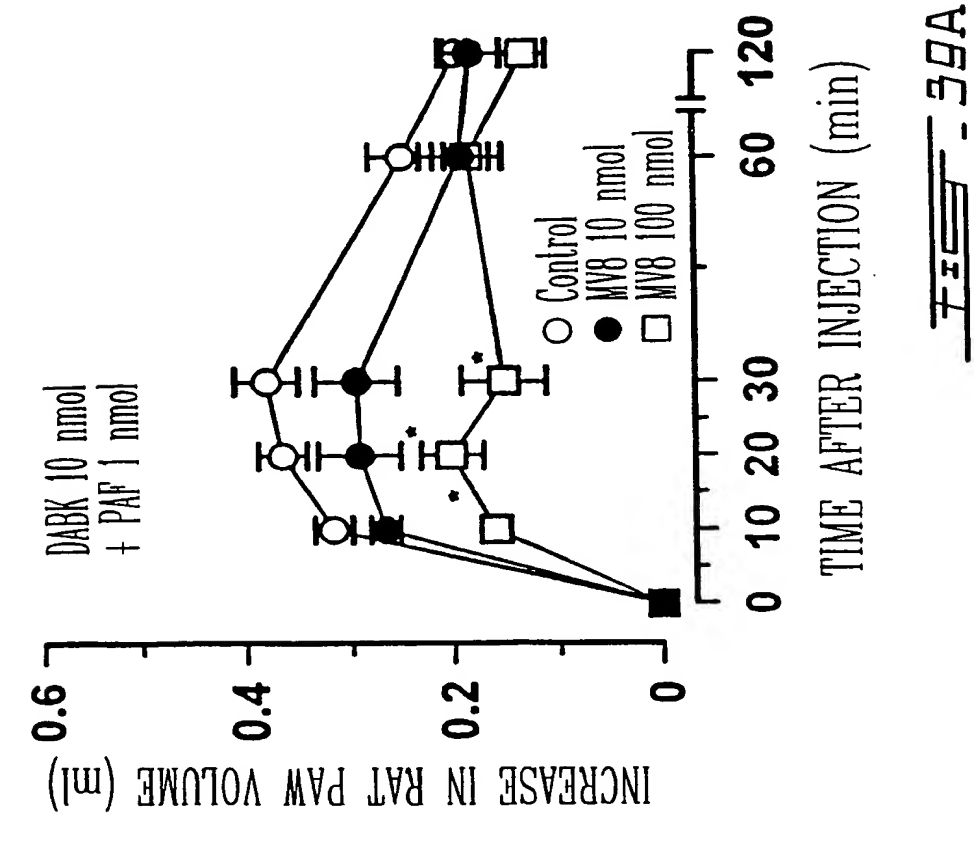


FIG - 39A

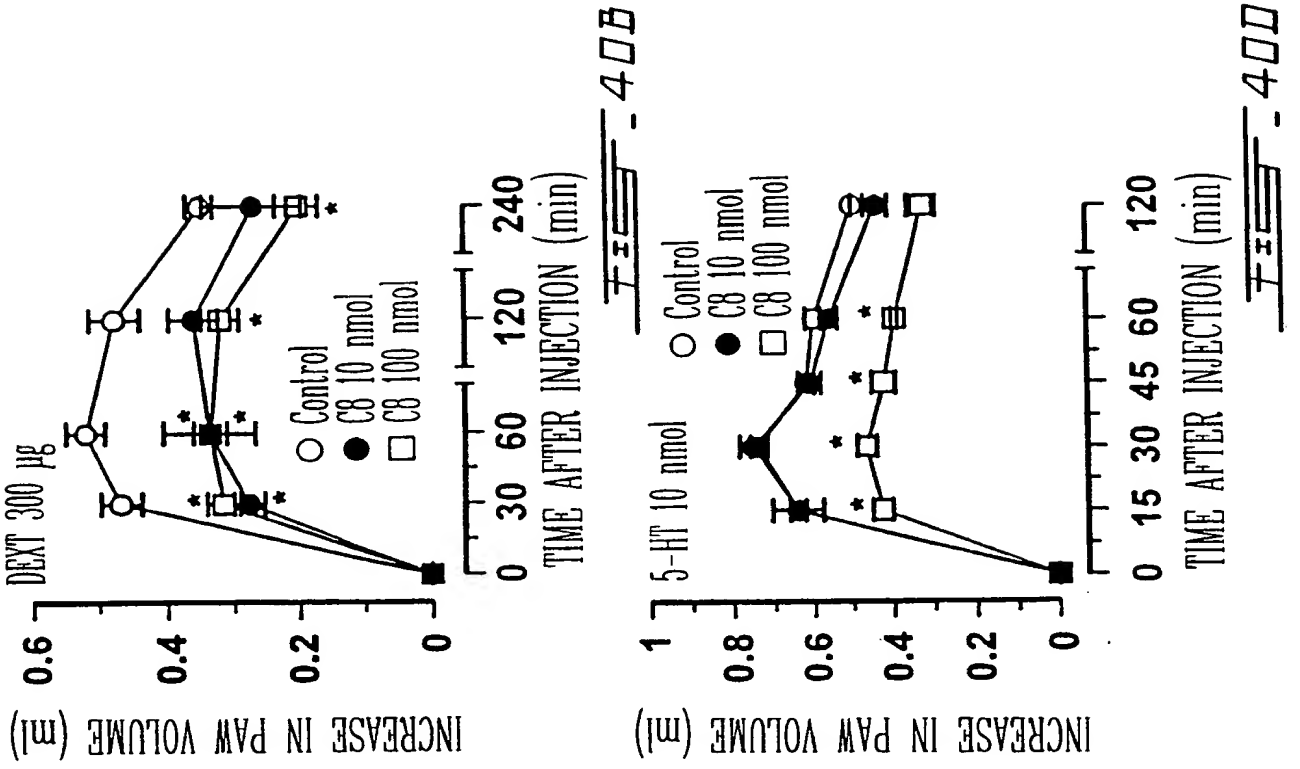


FIG. 40A FIG. 40B FIG. 40C FIG. 40D

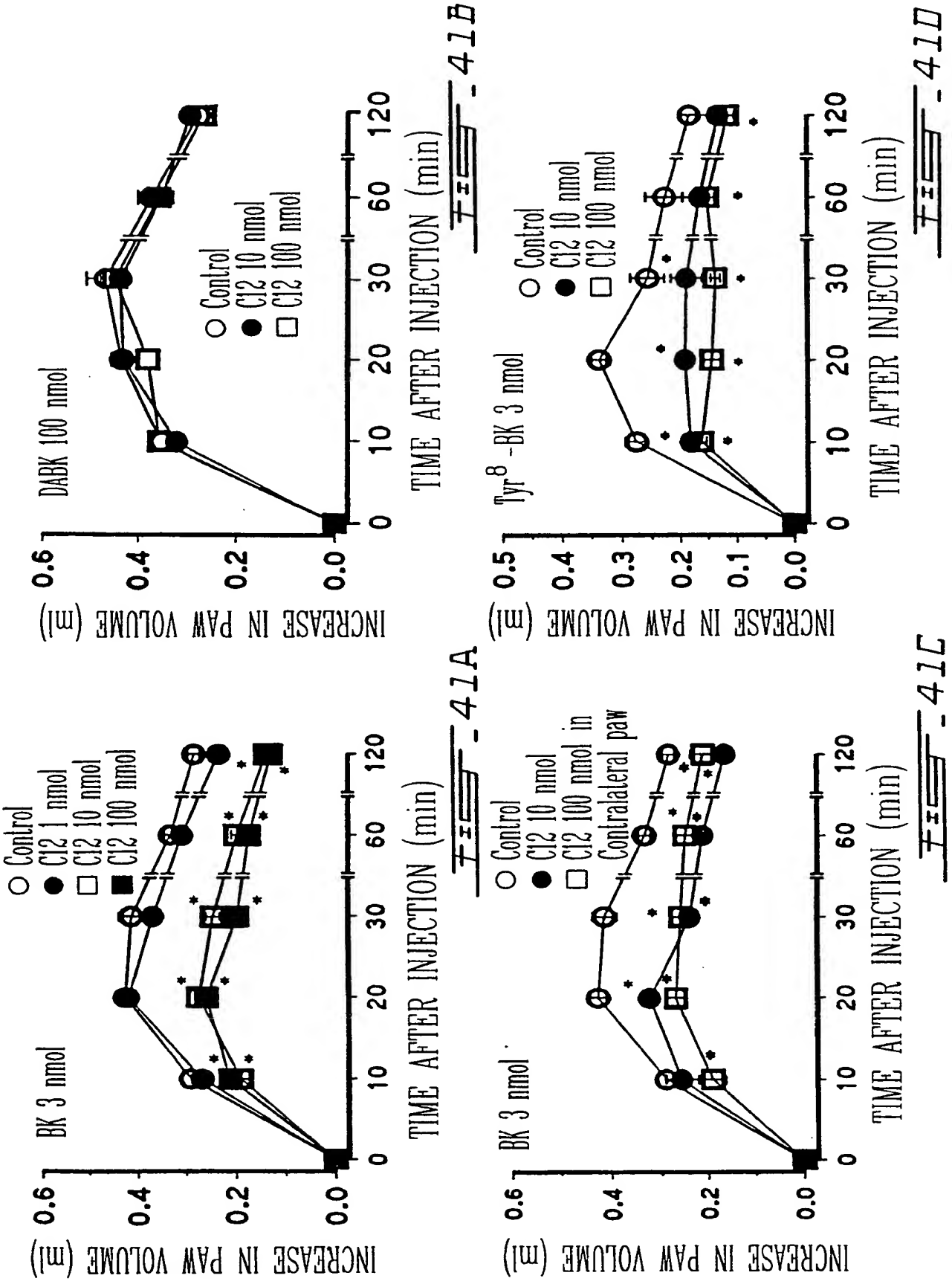


Fig-41A

Fig-41B

Fig-41C

Fig-41D

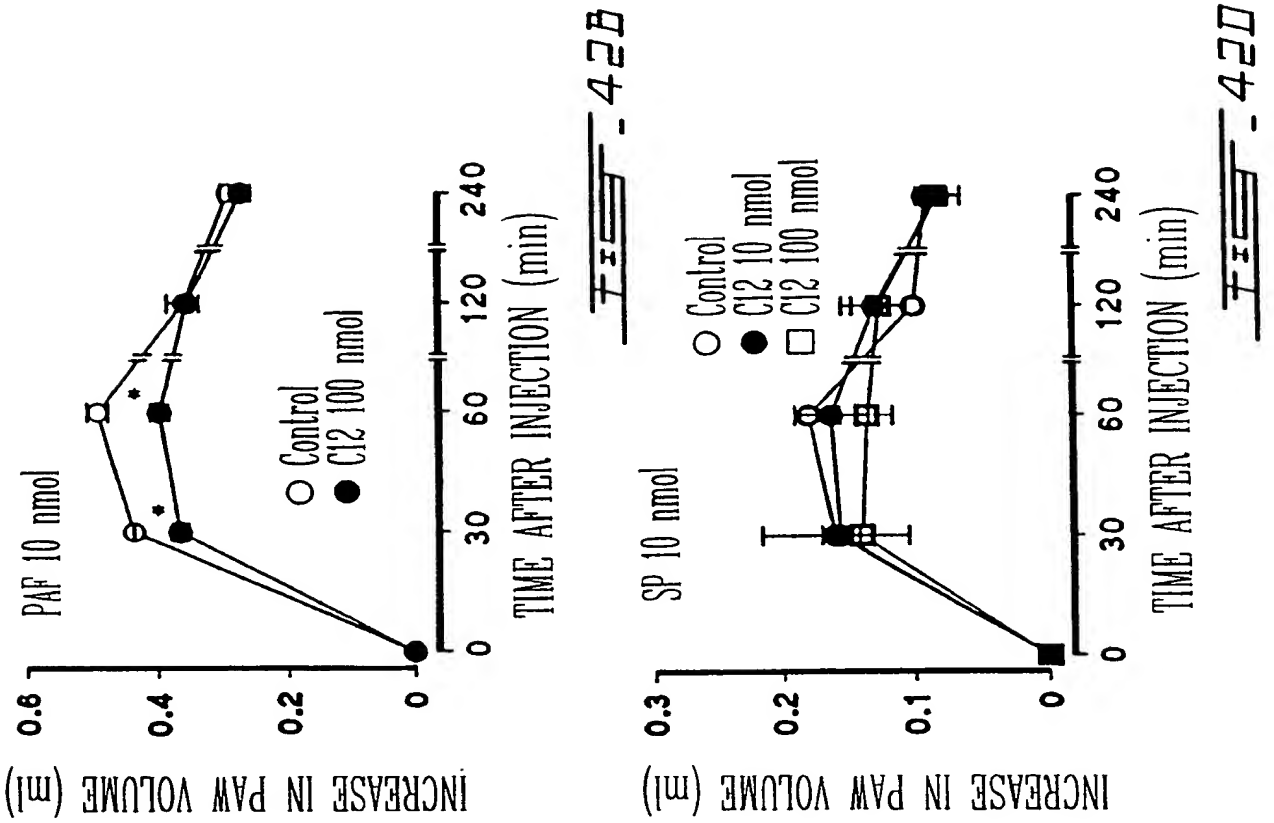


FIG - 42B

FIG - 42D

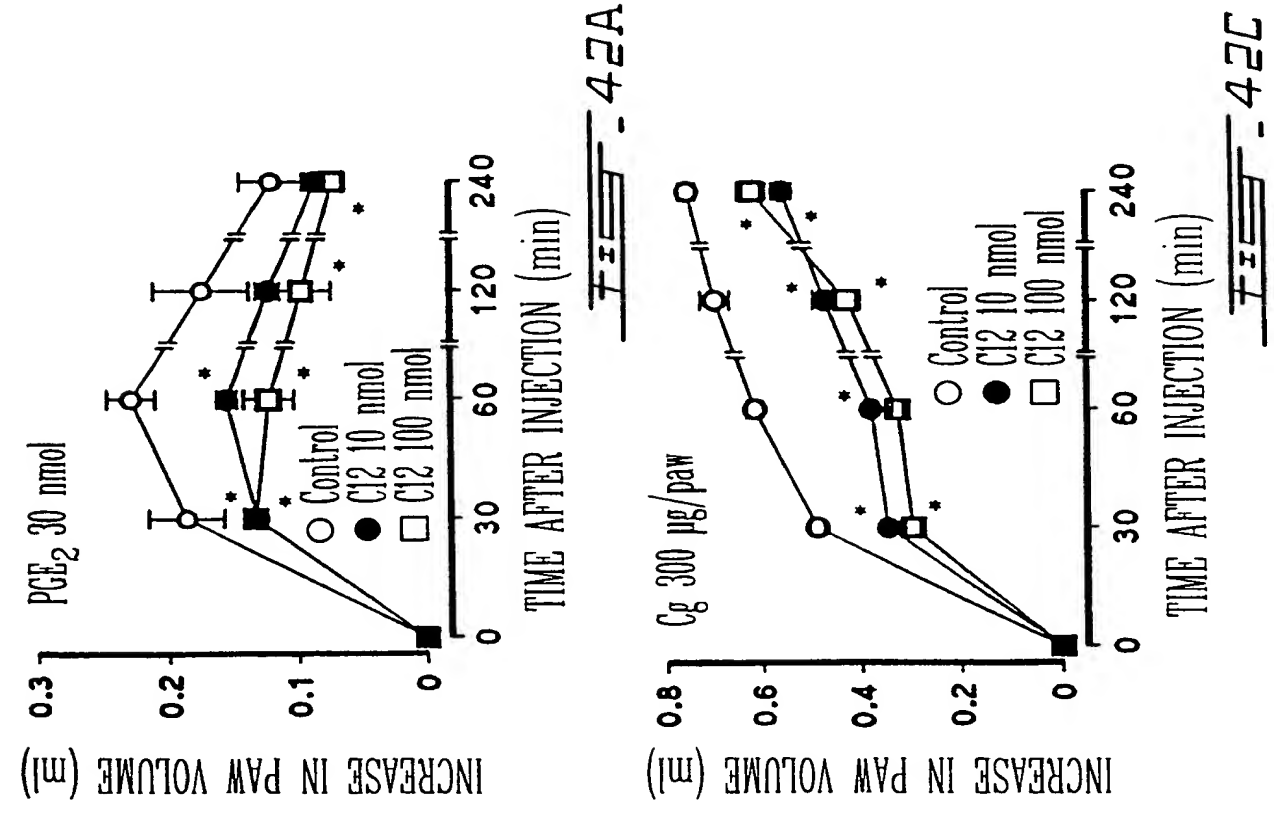
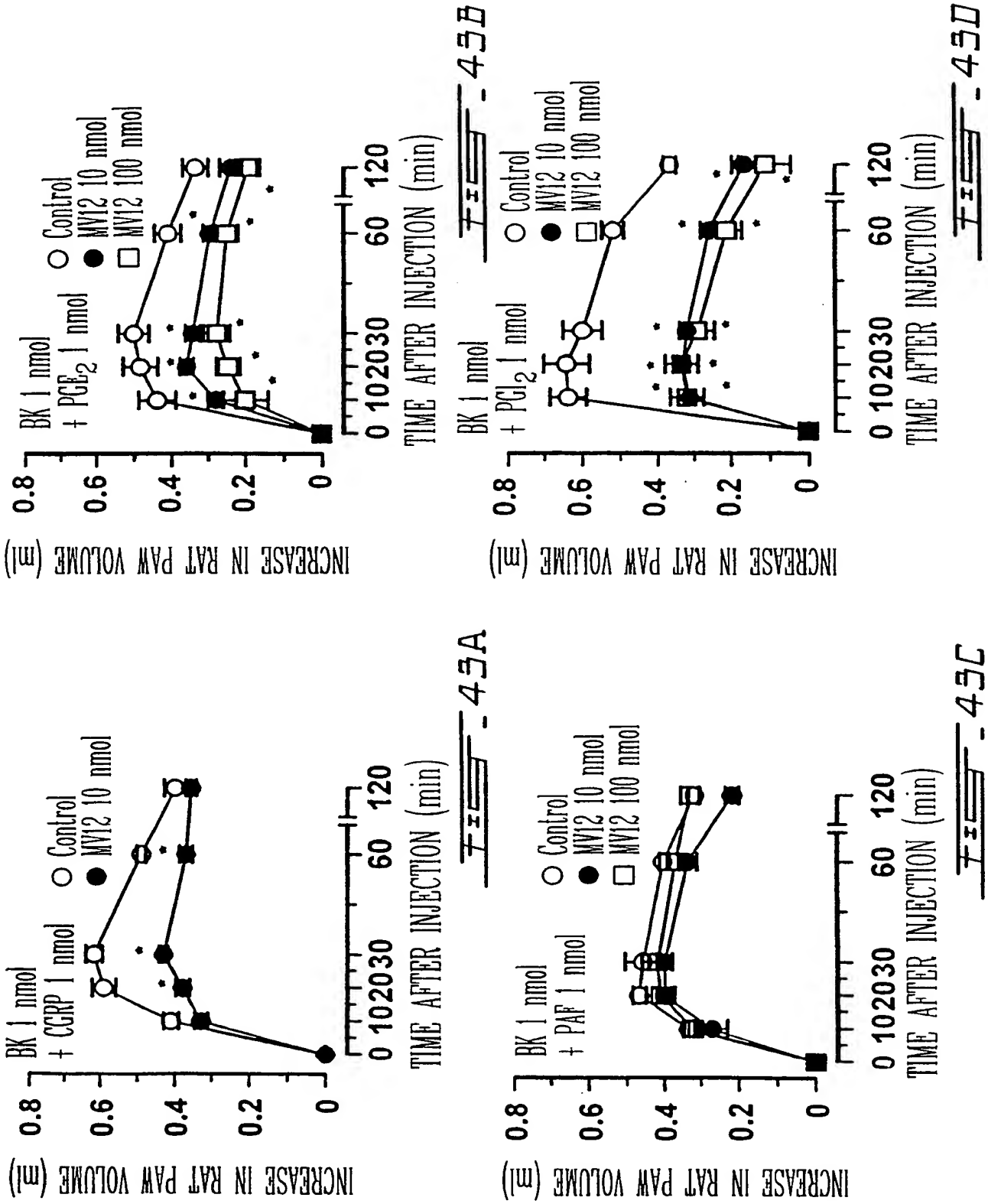


FIG - 42A

FIG - 42C


BK 1 nmol
+ CCRP 1 nmol
Control
MV12 10 nmol
INCREASE IN RAT PAW VOLUME (ml)
TIME AFTER INJECTION (min)
0 10 20 30 60 120
0 0.2 0.4 0.6 0.8
FIG - 43A
BK 1 nmol
+ PGE_2 1 nmol
Control
MV12 10 nmol
MV12 100 nmol
INCREASE IN RAT PAW VOLUME (ml)
TIME AFTER INJECTION (min)
FIG - 43B
BK 1 nmol
+ PAF 1 nmol
Control
MV12 10 nmol
MV12 100 nmol
INCREASE IN RAT PAW VOLUME (ml)
TIME AFTER INJECTION (min)
FIG - 43C
BK 1 nmol
+ PGI_2 1 nmol
Control
MV12 10 nmol
MV12 100 nmol
INCREASE IN RAT PAW VOLUME (ml)
TIME AFTER INJECTION (min)
FIG - 43D

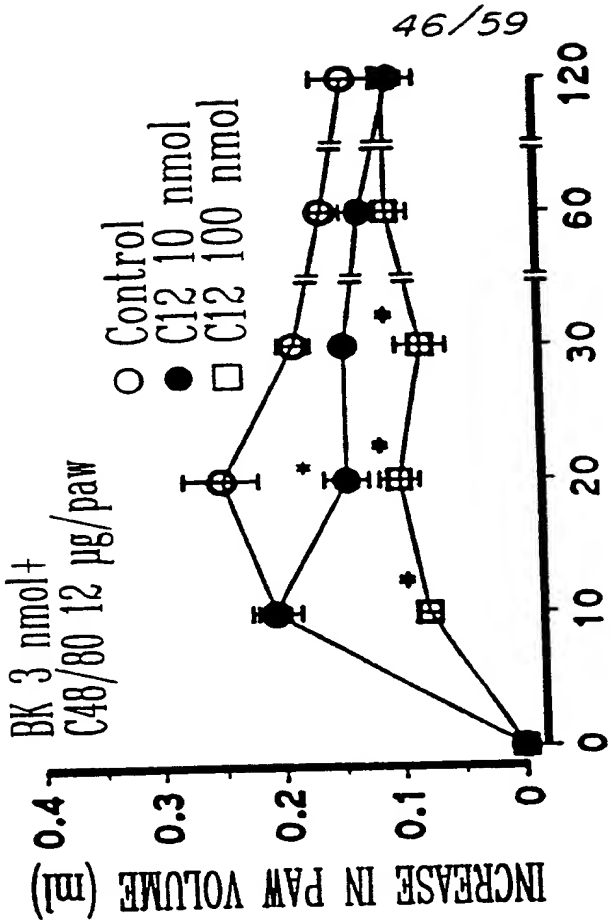


FIG - 44B

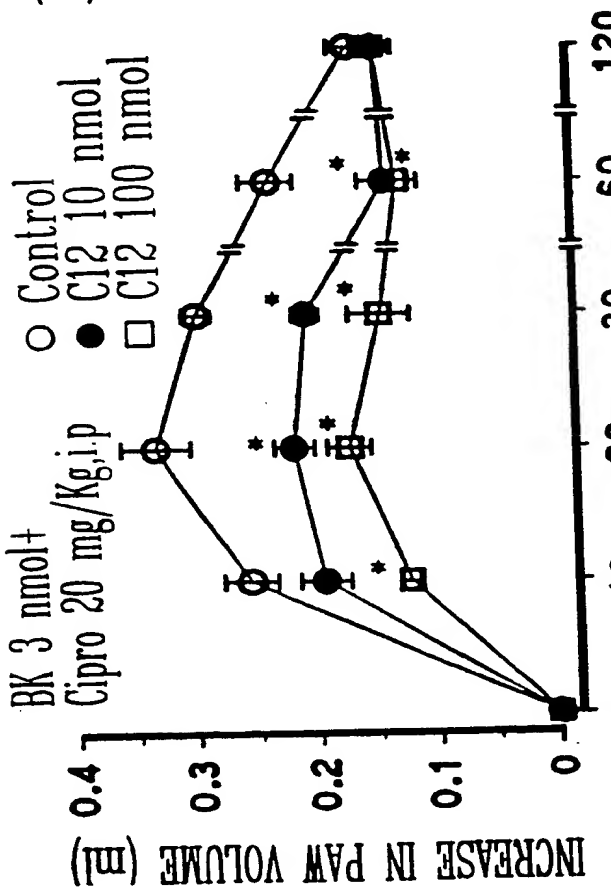


FIG - 44A

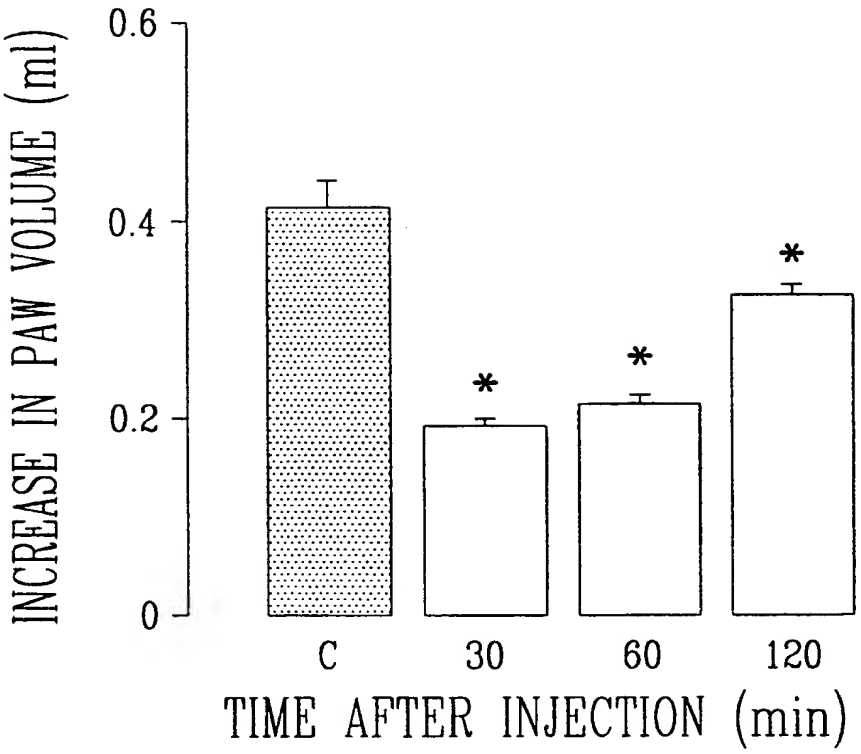

INCREASE IN PAW VOLUME (ml)
0.6
0.4
0.2
0
*
*
*
C
30
60
120
TIME AFTER INJECTION (min)

Fig. 45

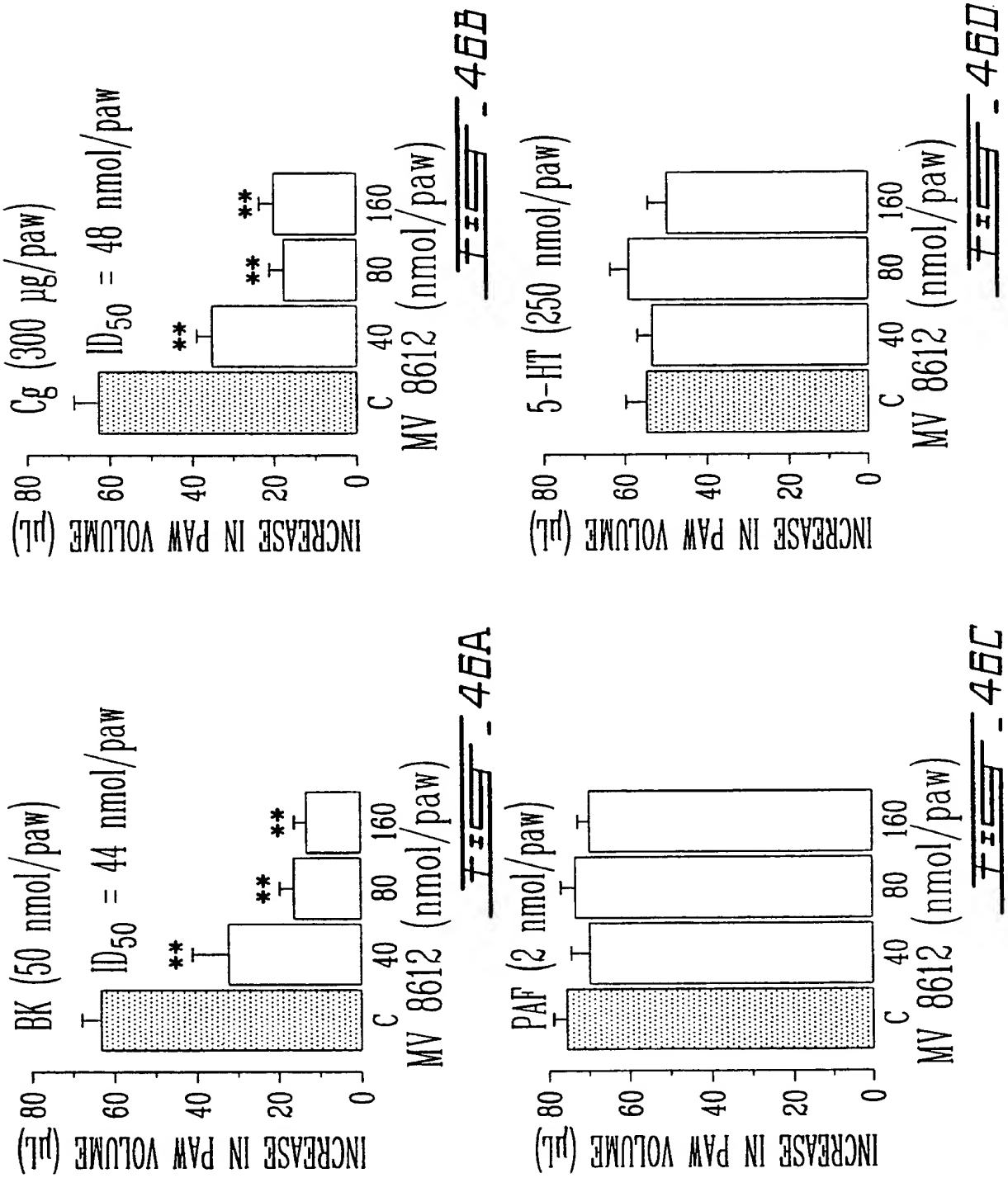


FIG - 4A

FIG - 4B

FIG - 4C

FIG - 4D

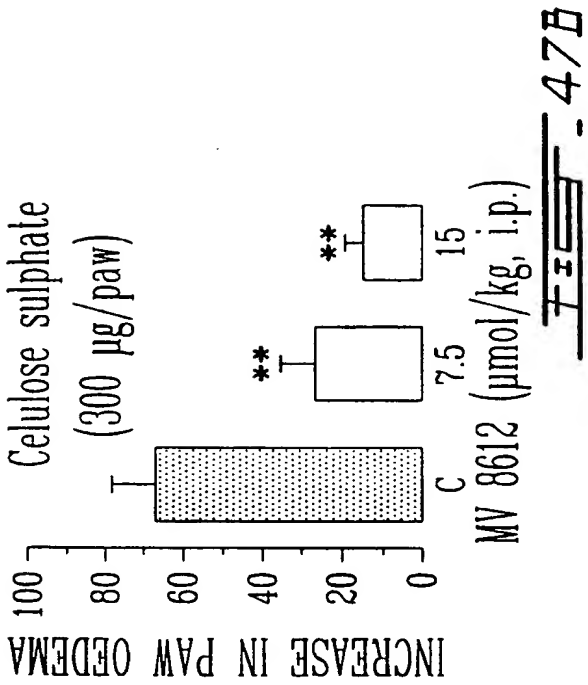


Fig - 47B

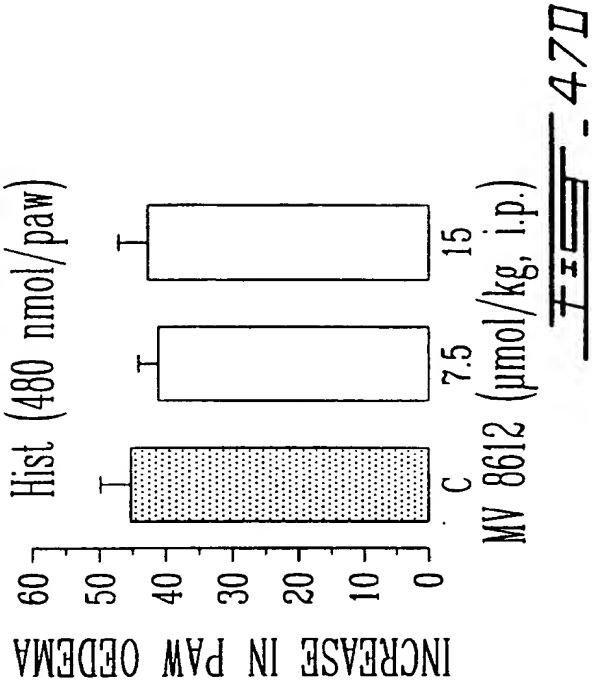


Fig - 47D

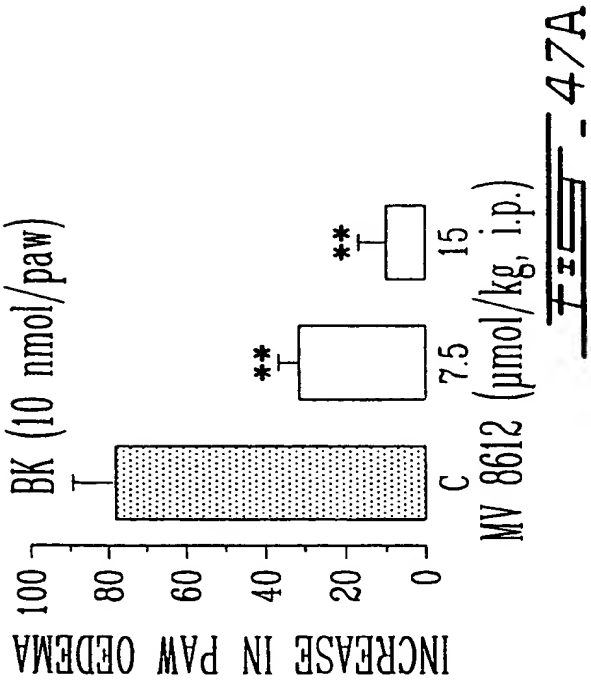


Fig - 47A

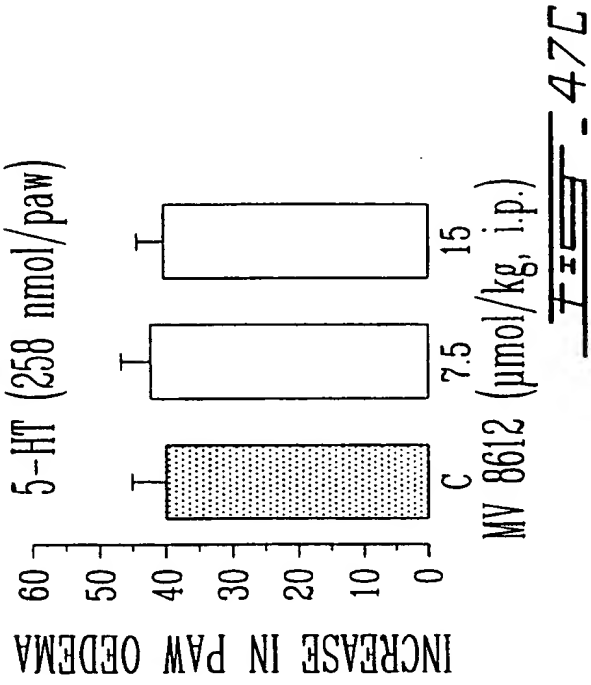


Fig - 47C

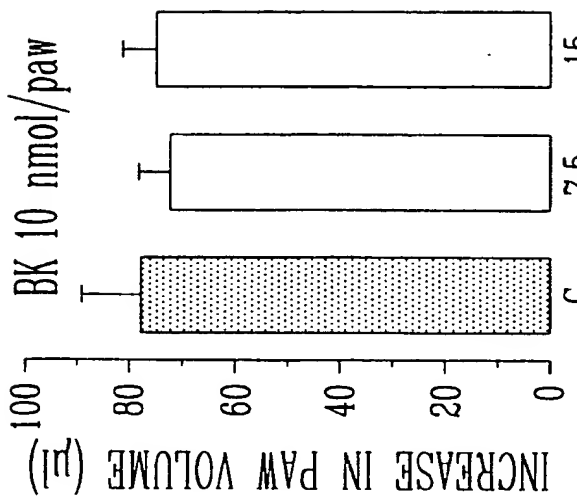


FIG - 4BA

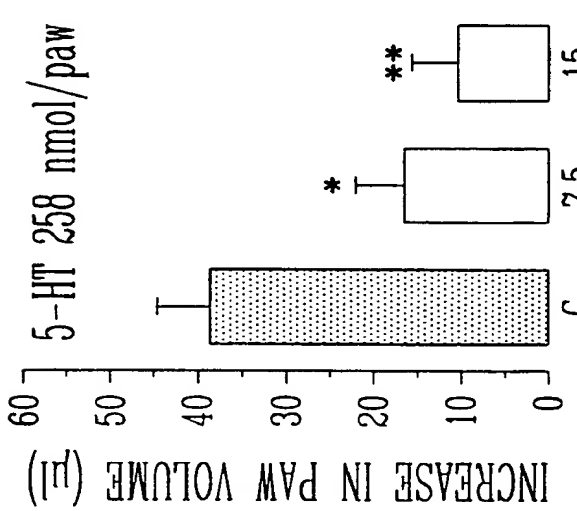


FIG - 4BB

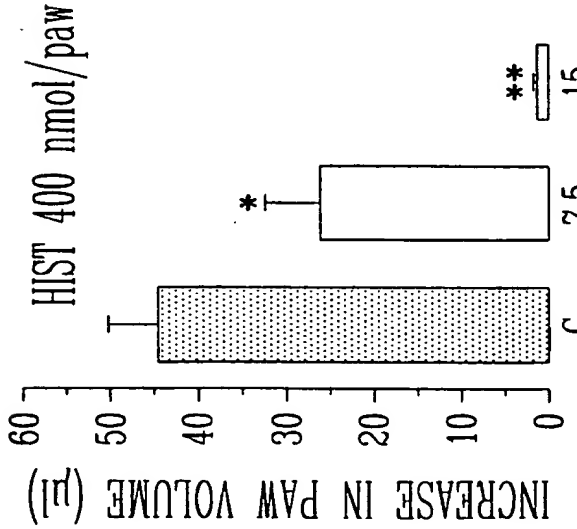


FIG - 4BC

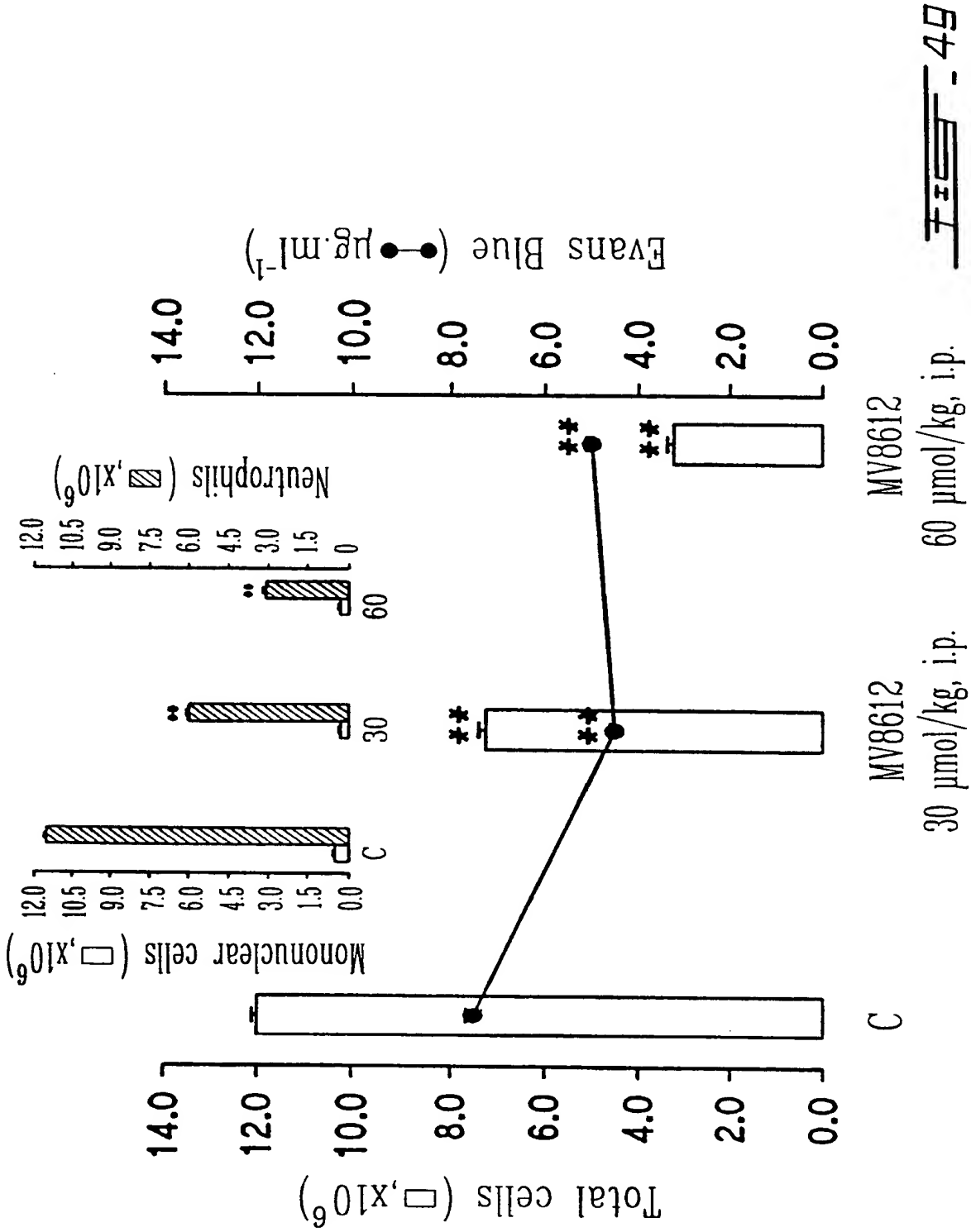

Total cells ($\square$,x10^6)
Mononuclear cells ($\square$,x10^6)
Neutrophils ($\boxtimes$,x10^6)
Evans Blue (●—●µg.ml^{-1})
C
MV8612 30 µmol/kg, i.p.
MV8612 60 µmol/kg, i.p.

Fig - 4g

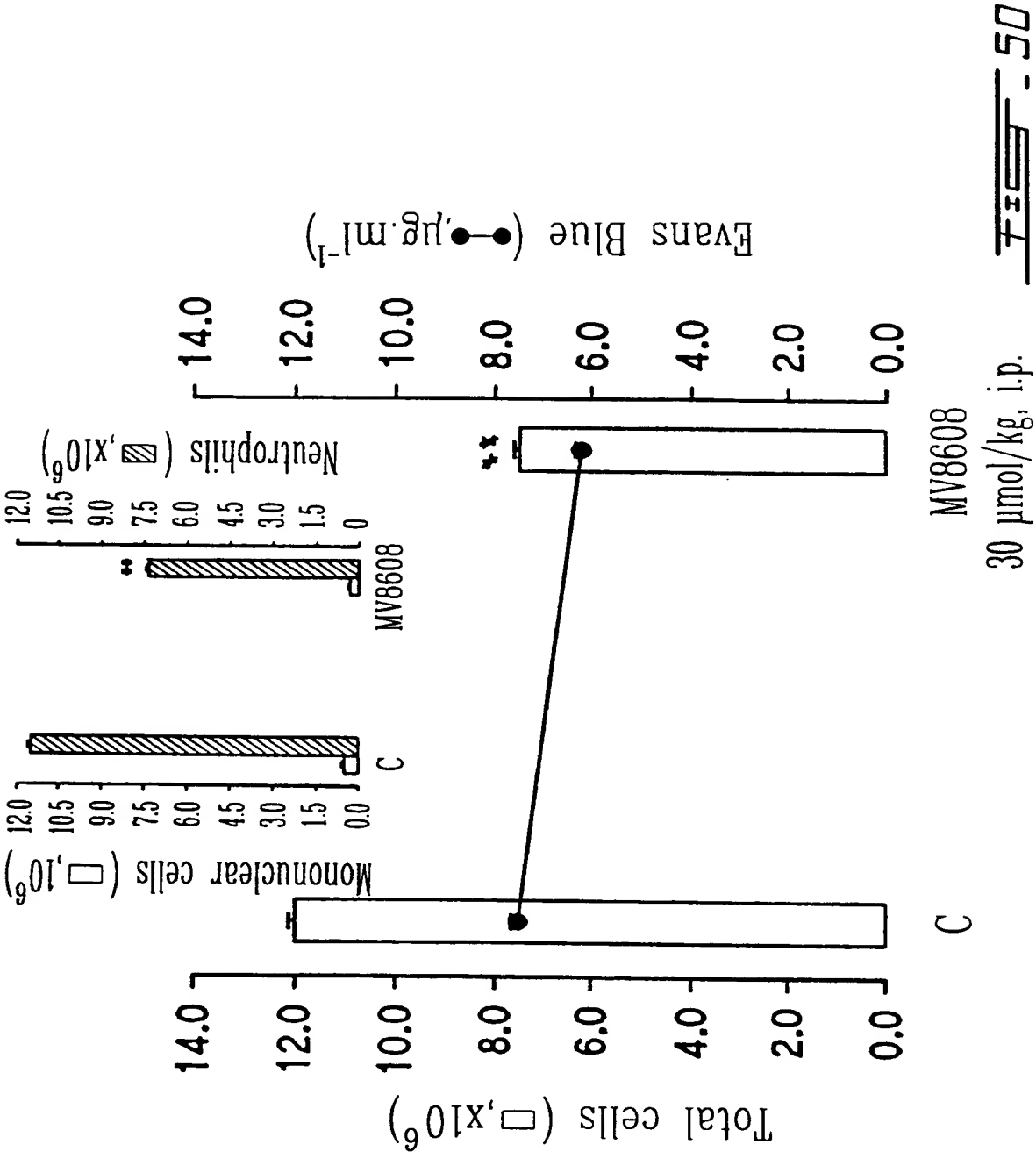

Total cells (□,x10⁶)
Mononuclear cells (□,10⁶)
Neutrophils (▨,x10⁶)
Evans Blue (●—●,μg.ml⁻¹)
C
MV8608
30 μmol/kg, i.p.

FIG - 50

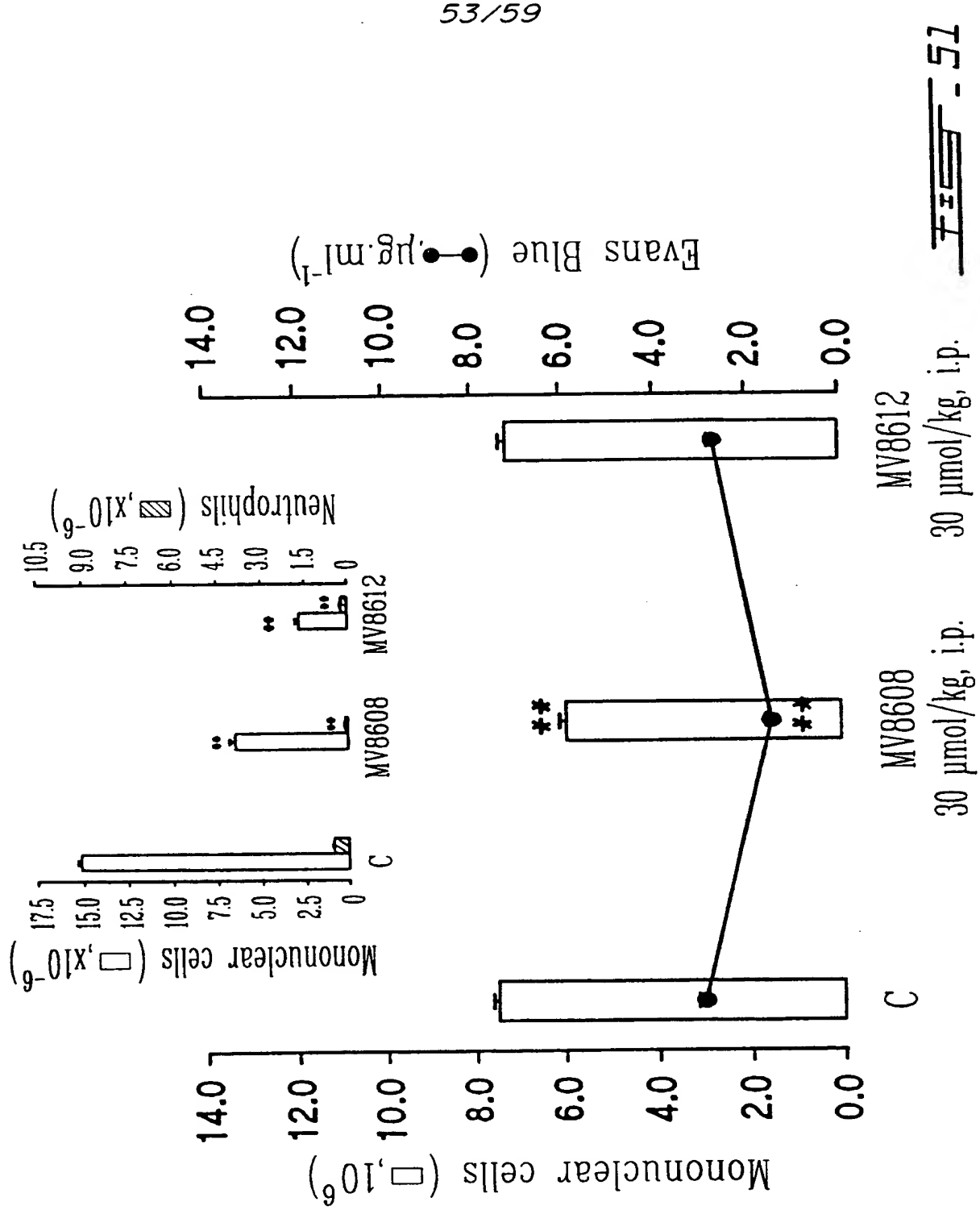

Evans Blue (●—●, µg.ml⁻¹)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Mononuclear cells (□, 10⁶)
Neutrophils (▨, x10⁻⁶)
10.5
9.0
7.5
6.0
4.5
3.0
1.5
0
Mononuclear cells (□, x10⁻⁶)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0
C
MV8608
MV8612
30 µmol/kg, i.p.
**

FIG - 51

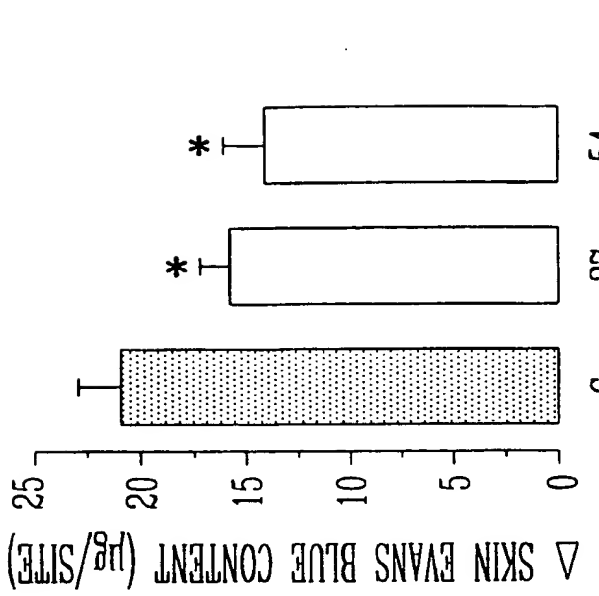


FIG - 52B

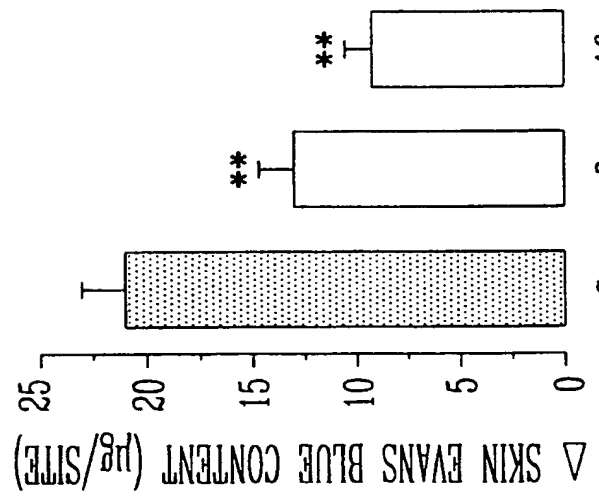


FIG - 52A

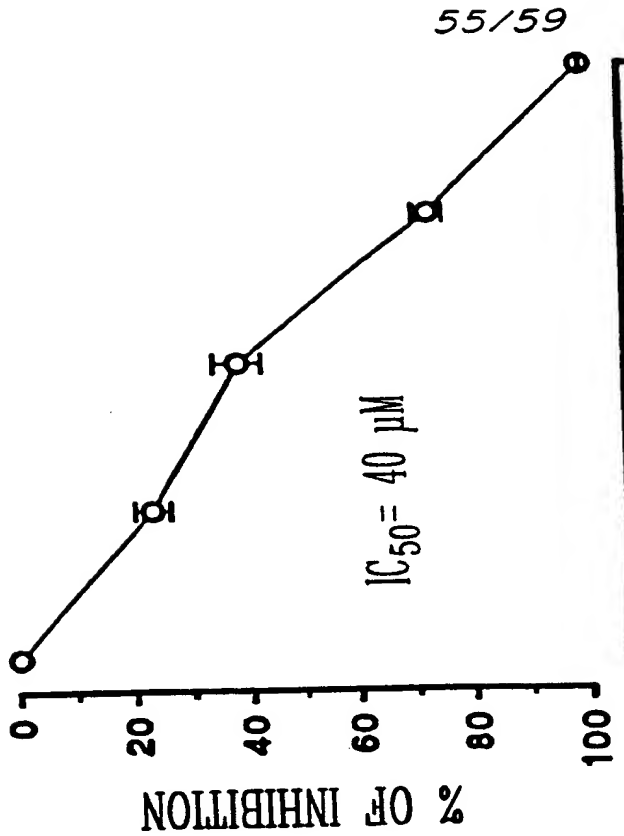


FIG - 53B

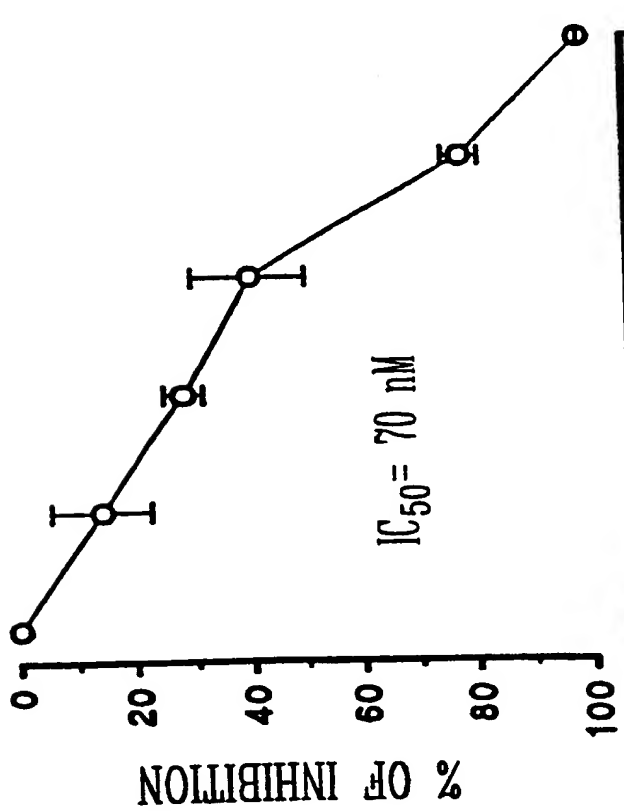


FIG - 53A

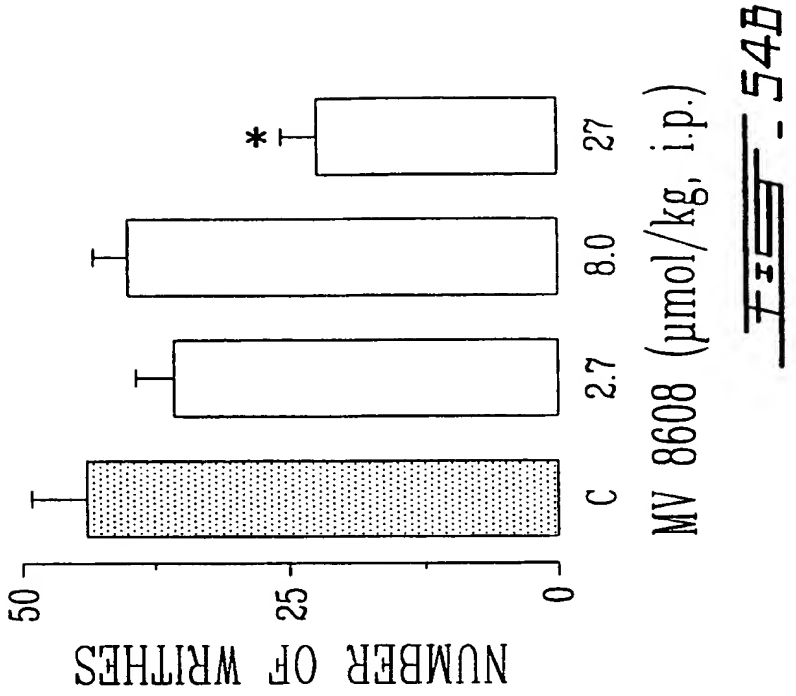


FIG - 54B

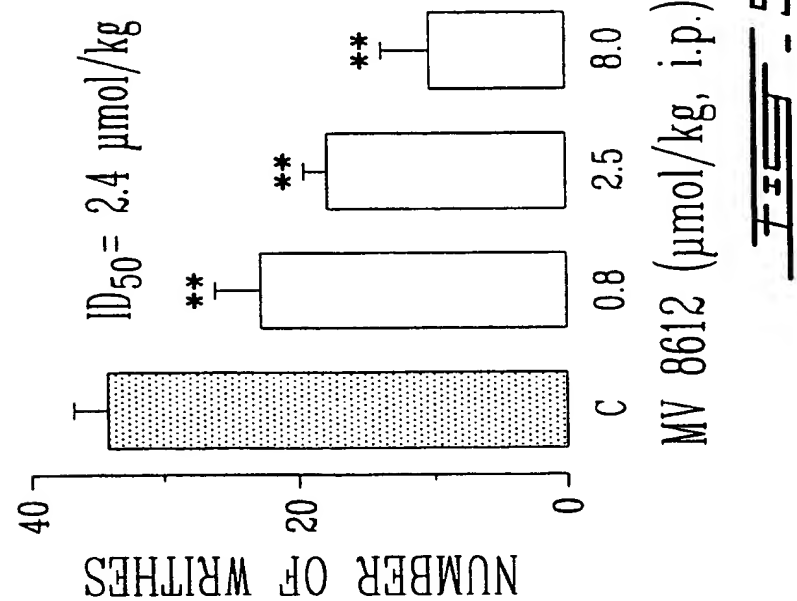


FIG - 54A

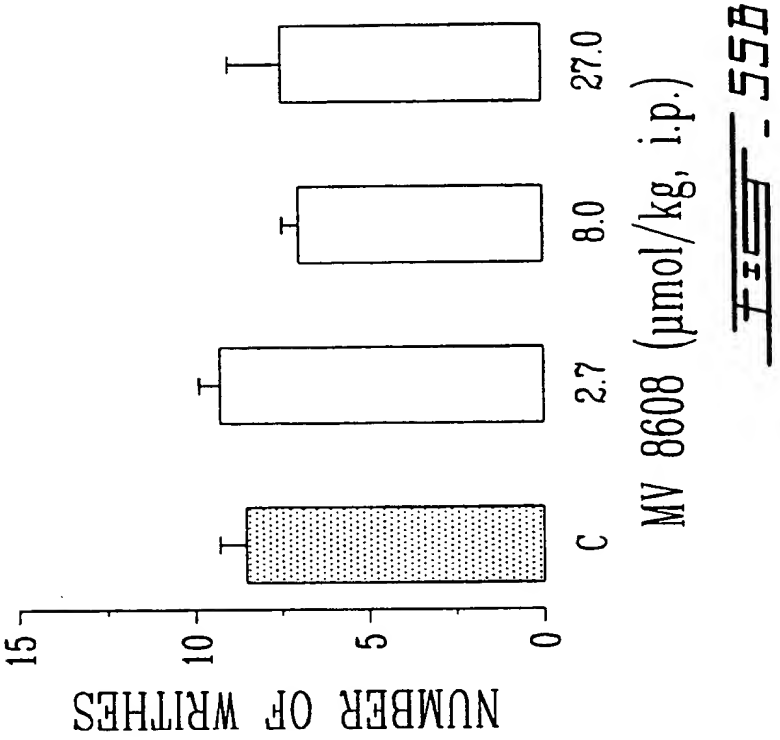


FIG - 55B

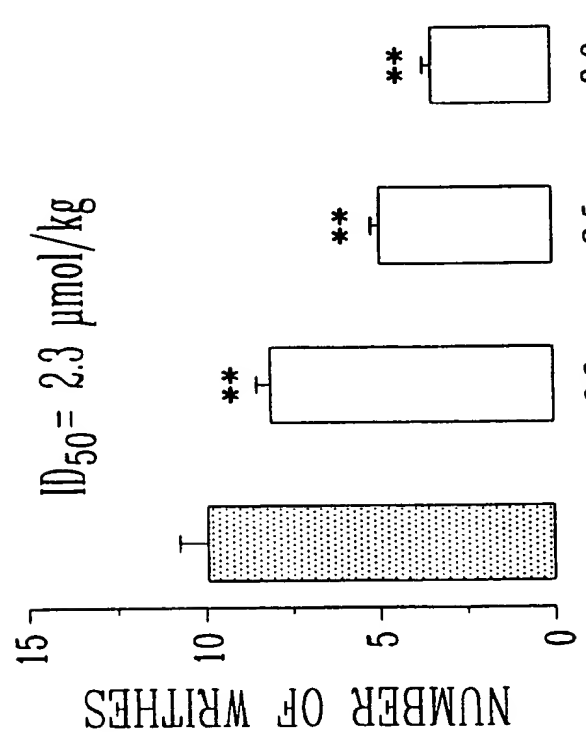


FIG - 55A

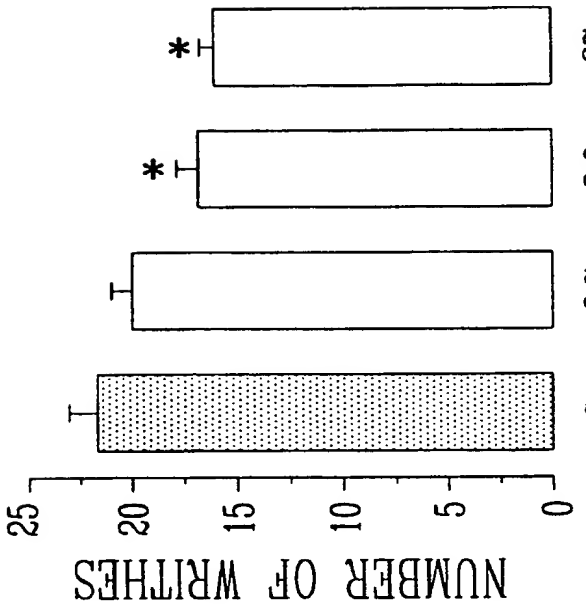


FIG - 5BB

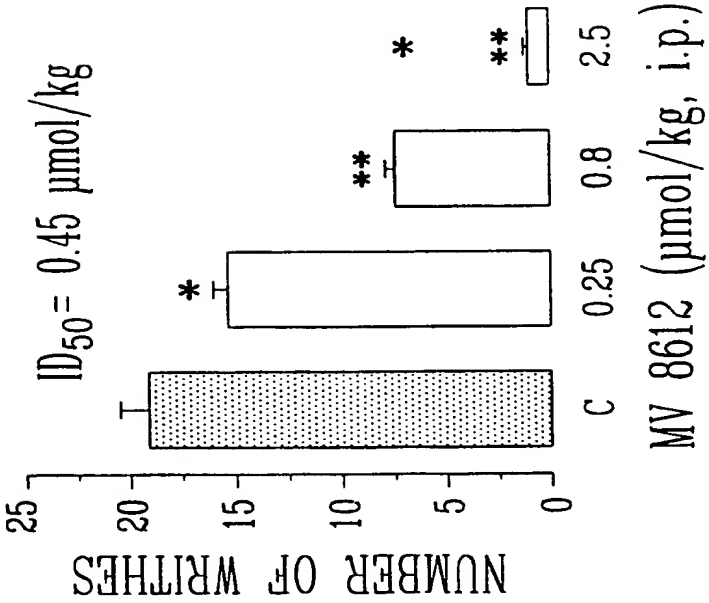


FIG - 5BA

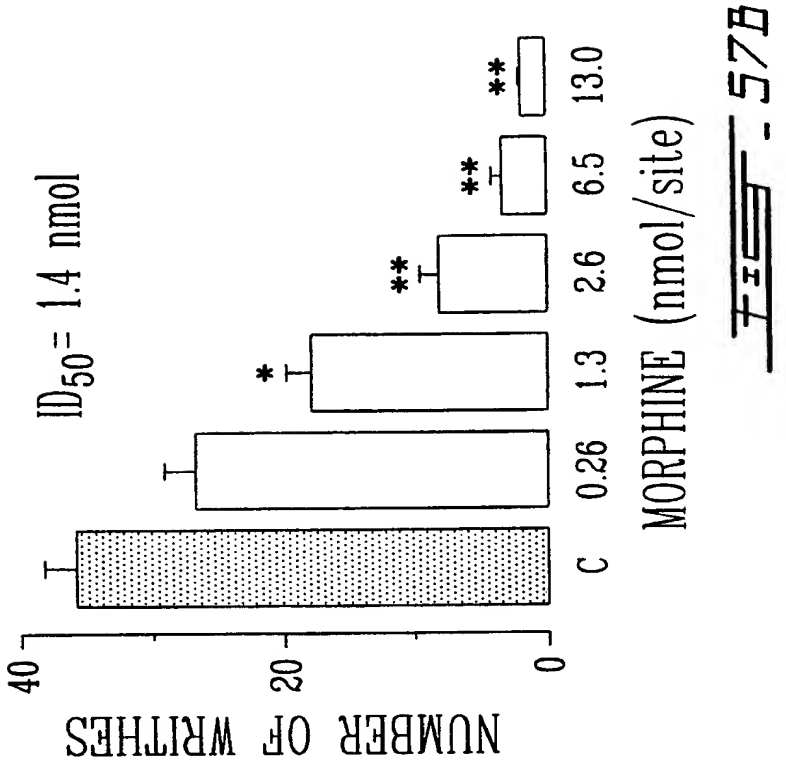


FIG - 57B

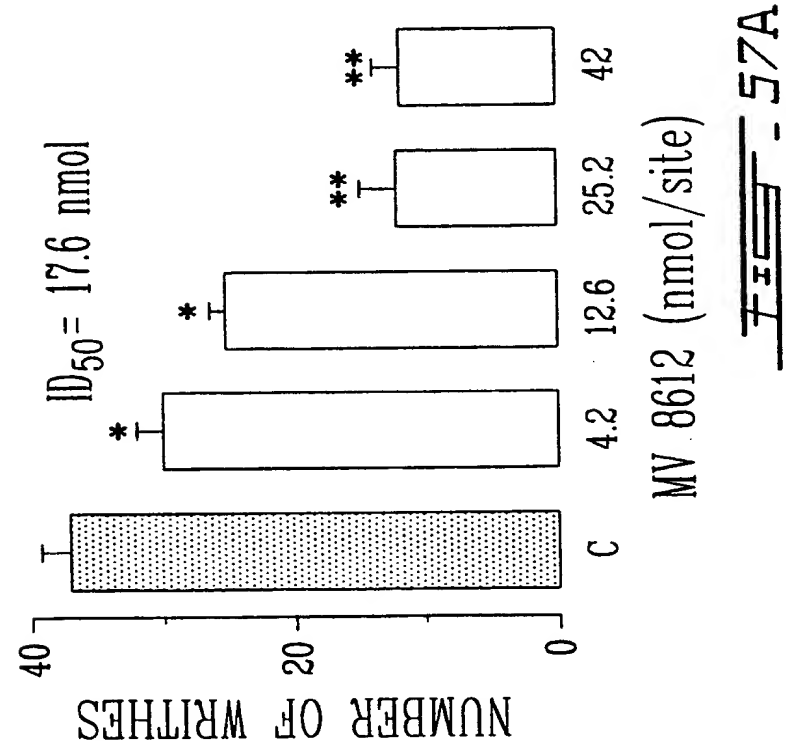


FIG - 57A

INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/CA 98/00908

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/58 C07J71/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	NEVES P C; NEVES M C; CRUZ A B; SANT'ANA A E; YUNES R A; CALIXTO J B: "Differential effects of Mandevilla velutina compounds on paw oedema induced by phospholipase A2 and phospholipase C. " EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 243, no. 3, 1993, pages 213-9, XP002092176 see the whole document --- -/--	1-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bardili, W

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 98/00908

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
X	BENTO, EDSON S.; CALIXTO, JOAO B.; HAWKES, GEOFFREY E.; PIZZOLATTI, MOACIR G.; SANT'ANA, ANTONIO E. G.; YUNES, ROSENDO A.: "The structure of velutinol A is (15R,16R,20S)-14,16:15,20:16,21-triepoxy-15,16-seco-14.beta.,17.alpha.-pregn-5-ene-3.beta.,15-diol. A combined quantitative Overhauser effect and molecular modeling study" J. CHEM. SOC., PERKIN TRANS. 2, no. 7, 1996, pages 1359-1366, XP002092177 see the whole document ---	1-17,26, 27
Y	EP 0 359 310 A (PROCTER & GAMBLE) 21 March 1990 cited in the application see page 1 - page 2 ---	1-27
X	BEYER: LEHRBUCH DER ORGANISCHEN CHEMIE, 1981, page 658 XP002092178 see 'pregnenolon' -----	9

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INTERNATIONAL SEARCH REPORT

.nformation on patent family members

Inter ›nal Application No
PCT/CA 98/00908

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0359310 A	21-03-1990	JP 2142736 A	31-05-1990